

Theory of Growth and Competition in Natural Populations

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ABSTRACT

Classical theories of population growth and inter-species relations are briefly reviewed. It is shown how variations in feeding habits, reproductive patterns, and other aspects of population behaviour have required modifications of the basic theory in order to make it applicable to particular species. The role of the environment in population theory is also discussed, and this opens the way to a consideration of theoretical aspects of the ecology of natural populations. The latter are admittedly too complex to encourage the application of theory in its purest and most complete form. Practical attempts along these lines have simplified the problem by dealing with ecological groups—phytoplankton, zooplankton, etc.—rather than individual species. A review is presented of the methods, aims, and results of theoretical ecological investigations of the last few years. The significance of such work with respect to higher members of the food chain is discussed, and the paper concludes with remarks on some of the unsolved problems of theoretical analysis.

INTRODUCTION

DURING the last thirty years, there have been rapid advances in theories of population growth and inter-species relations. The work of Lotka, Volterra, and Gause—to mention only a few of the most important contributors—has resulted in a precise, mathematical formulation of growth problems which has stimulated experimental examination and has helped biologists to think more clearly about their results. The function of biomathematics is to relate in exact, quantitative terms the history of a population that has a certain group of arbitrarily defined biological properties. This can be a useful tool for testing our theories about the nature of these properties. However, it is obvious that biomathematics does not wave a magic wand over a sheet of graph paper. Its applications have been only partially successful, because the results are no better than the biological knowledge of the mind that conceives the theory.

The simplest way to study a population and to attempt to apply the rigid criteria of mathematical analysis is through the agency of a controlled laboratory experiment. However, it was inevitable that naturalists and ecologists would soon take note of the mathematics of population behaviour and would begin to wonder if there was a method that would help them to understand the difficult and often baffling array of species relationships that they observe in nature. The terminology and concepts of the theorists gradually infiltrated ecological discussions during the 1930's and 1940's. The sigmoid growth curve, the niche concept, prey-predator oscillations, and other essentially mathematical ideas have become part of the stock in trade of the ecologist. They have been used, reused, and sometimes misused. The latter is inevitable, for it is often very difficult to

decide whether the basic assumptions used in formulating the equations are applicable to a particular ecosystem.

The initial assumptions are essentially generalizations upon the biological facts of a population: rates of feeding, growth, and reproduction, and variations in these processes in response to environmental changes; food preferences; methods of avoiding predators. The ecologist is well aware of the variety of life habits in nature and the difficulty of generalizing upon them. Perhaps he is not quite so aware of the ways that the mathematical results are altered by small changes in these assumptions. It therefore seems worth while to review the elements of classical population theory from an ecological point of view and to show how the theory may be modified to make it applicable to particular problems of population growth in nature.

ECOLOGICAL ASPECTS OF POPULATION GROWTH

There is a basic premise that has served as the departure for nearly all growth theories. It states that *in an unlimited environment, the multiplication of organisms proceeds as a geometrical progression*. In the language of differential calculus, the rate of change of the number of organisms N with respect to time t may be written

$$\frac{dN}{dt} = bN \quad (1)$$

where b is a growth coefficient, meaning, in arithmetic terms, that the percentage increase in the population during successive, equal intervals of time is a constant. If there are no environmental restrictions whatever, the growth coefficient is an innate characteristic of the species. It may vary enormously from one species to another, as when we compare bacteria and human beings. But the ecologist is quick to point out that even in a hypothetical environment that is unlimited with respect to food and space, the value of b for a given species will not be invariable. It will be conditioned by temperature and other physical factors. Conditions that are optimal for one species will be suboptimal for another. Thus there is an ecology in the simplest kind of environment we can conceive.

A second premise states that *in a limited environment there is a maximal number of individuals which cannot be surpassed*. Ordinarily as the number of individuals approaches the maximum, multiplication proceeds more slowly. Thus the curve of population growth is sigmoid. Mathematically it can be expressed in several ways. One example is the logistic of Pearl and Reed (1920). The differential equation is

$$\frac{dN}{dt} = bN \cdot \frac{K - N}{K} \quad (2)$$

where K is the maximum population. When N is very small, the growth rate approaches the geometrical progression postulated by equation (1). As the number of individuals approaches the maximum number K , the growth rate approaches zero, and the population curve levels off. The two types of curves, the geometrical progression and the logistic, are schematically shown in Figure 1.

The logistic is essentially empirical. In a way that is more or less consistent with observed population-growth curves, it expresses the idea that something limits growth. It leaves the biologist free to interpret the limitation. If the latter is simply a matter of available space, certain chemical or physical factors become increasingly unfavourable as the population grows. Metabolic products accumulate. In the case of autotrophic plants, the increase in the density of the population reduces the amount of light available for each organism. Alternatively, an insufficient supply of food or other essential substances may stop growth at a still lower level. Thus the K as well as the b in the equation is an ecological variable.

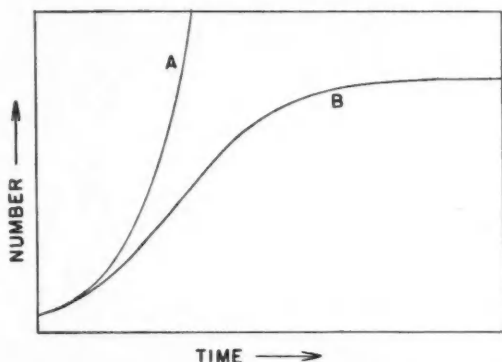


FIGURE 1. Theoretical growth curves. A. Geometrical progression, the integral form of equation (1). B. The logistic, the integral form of equation (2).

The logistic presents in ideally simple form some of the major aspects of the growth process. It falls short of a complete explanation, and therefore it is not surprising that other types of premises, and other families of sigmoid curves, have also been applied to the problem. Moreover, the biological properties of some species depart so far from the conditions specified that the population curve deviates from the sigmoid form. Bacterial growth commonly resembles the logistic in the early stages, but instead of arriving at a level phase—an upper asymptote—the organisms often die rapidly or are transformed into spores. Diatoms and other algae are also likely to increase to a peak and then decline, but they tend to establish an equilibrium population at some lower level. In *Daphnia* populations, which behave similarly, Slobodkin (1951) has demonstrated by a quantitative, theoretical analysis that the form of the curve results from changes in the physiological properties of the animals with age. The coefficient b is not a simple constant; the age and size structure of the population introduce additional requirements that must be fulfilled by altering the mathematical treatment. The other examples have not been carefully examined. It suffices for the moment to point out the danger of discussing the ecology of a variety of species in terms of any one type of growth curve, which is known to have only limited validity.

ECOLOGY OF INTER-SPECIES RELATIONS

Gause (1934) applied the logistic to a consideration of inter-species relations. By mathematical and experimental methods, he demonstrated that when two species compete for the same food in a limited environment, the growth rates of both will be reduced, and in most cases one species will eventually eliminate the other. The equations for the growth rates were written

$$\frac{dN_1}{dt} = b_1 N_1 \cdot \frac{K_1 - (N_1 + \alpha N_2)}{K_1}, \quad (3)$$

$$\frac{dN_2}{dt} = b_2 N_2 \cdot \frac{K_2 - (N_2 + \beta N_1)}{K_2}, \quad (4)$$

The subscripts 1 and 2 serve to distinguish the two species. The equations are the same as the logistic except that the terms αN_2 and βN_1 are inserted to indicate the degree of influence of each species on the other. Each one captures part of the food or other environmental necessities that otherwise would go to its competitor. Neither is immediately able to achieve the population size that would be possible if the other species were absent. Nor is it likely that an equilibrium population will be obtained until one species is eliminated. A two-species equilibrium requires, by definition, that

$$\frac{dN_1}{dt} = \frac{dN_2}{dt} = 0,$$

from which it is apparent that the right-hand members of the equation also must equal zero. This requires an exact balance of biological processes that apparently has never been achieved in a laboratory experiment. If the growth rate of N_1 is slightly greater than that of N_2 , βN_1 will increase faster than αN_2 , and the difference between the growth rates will be further increased. It is a relation that always tends to move away from equilibrium at increasing speed, a process that can only end in rendering one species extinct.

The ecologist, confronted with the reality of the coexistence of dozens of species with a similar mode of life, has nevertheless accepted the competition theory with a reverse twist. It has become a truism that no two species occupy the same niche; i.e., their food or their habitat are never completely identical, so that competition exists, with varying degrees of severity, but seldom to the point of rendering a species extinct. The niche concept has been defined so broadly that it often literally means anything—usually an undetermined something—that prevents the competition equations from operating according to plan. Several quite different types of behaviour will negate the equations. For example, (a) two species, living on the same general range of food species, have slightly different preferences (b) they have the same preferences, but the inferior species can exist on a less choice food, or (c) can adapt itself to a less favoured habitat; (d) they exist on exactly the same food, but variations in the physical environment alter their relative feeding efficiency. It is not unreasonable to postulate a situation in which the growth coefficient b_1 is larger than b_2 in summer and smaller in winter. This introduces a tendency toward a seasonal oscillation in

the abundance of the two species. If, in addition, each species is able to promote survival during the unfavourable season by resting stages or some other form of reduced metabolism, it will be impossible for one species to eliminate the other. Such occurrences may be partly responsible for the well known but little understood phenomenon of seasonal succession.

The relationship between predators and prey has been analysed by various theoretical biologists. Volterra's equations will serve as an introduction to the problem. Continuing the notation used previously, N_1 will be defined as the number of prey and N_2 the number of predators. The equation for the rate of change of prey with respect to time is

$$\frac{dN_1}{dt} = N_1 (b_1 - k_1 N_2), \quad (5)$$

where b_1 is the growth coefficient of the prey, and k_1 is a predation coefficient. The latter essentially means that a predator "hunts" a constant amount of space in unit time, consuming all of the prey therein. This assumption about feeding habits is probably justified in the case of filter feeders and some of the most rapacious selective predators. It is an oversimplification of the behaviour of highly organized creatures that modify their feeding habits according to metabolic needs. The remainder of the expression is identical with equation (1). In other words, predators provide the only check on unlimited geometrical increase. This is also a simplification of the facts in most cases.

Volterra's equation for the growth rate of the predators is

$$\frac{dN_2}{dt} = N_2 (k_2 N_1 - d_2), \quad (6)$$

where k_2 is a coefficient of food utilization, which may be synonymous with the coefficient of capture or may be some constant fraction of it; d_2 is a coefficient of death, ordinarily regarded as constant.

The interaction of the two equations is readily apparent. If the prey increase, the food utilization of the predators increases proportionally, so that the number of predators becomes larger. This in turn reduces the growth rate of the prey. Hence the population of prey reaches a peak and begins to decline more and more rapidly to a point where food capture is insufficient to maintain the stock of predators, and they too begin to decrease. The net result is a permanent oscillation of the two populations.

Ecologists have sometimes postulated that the Volterra equations explain population fluctuations of an apparently cyclical type that have been observed in nature. Proof or disproof is difficult, but the biological assumptions in the theory are too simple to inspire much confidence in its general applicability. No criticism of Volterra is implied. His equations seem to be basically sound. The terms can be modified in various ways to suit the requirements of a particular biological association. This is preferable to a more elaborate and less flexible system.

Two examples will illustrate the necessity of examining the basic assumptions

and modifying the treatment accordingly. Gause (1934) conducted a series of experiments in which the protozoan *Didinium nasutum* preyed upon *Paramecium caudatum*. No cyclical oscillation was obtained. *Didinium* did not die at a constant death rate d_2 , nor did the reproductive rate decline as the food supply diminished. The animals continued to increase rapidly in number, but with decreasing size. They were able to maintain a rate of food capture that eventually annihilated the prey. Gause rewrote the equations with more realistic assumptions, and the subsequent mathematical analysis verified and amplified the experimental results.

A second example is chosen from nature. In waters of the temperate zone, there is usually a short, spectacular burst of plant growth in late winter or early spring, the so-called spring diatom flowering. In many localities the zooplankton also increases during the flowering, reaching a peak at a later time while the plant population is declining. In a paper on phytoplankton-zooplankton relations, which has become a classic of biological oceanography, Harvey, Cooper, LeBour, and Russell (1935) described this spring growth period in the English Channel and presented convincing evidence that the zooplankton terminated the flowering by its grazing activities. Here was a phenomenon resembling one cycle of a Volterra oscillation. But after one cycle, a quasi-equilibrium was established. If there were any further oscillations, they were so thoroughly damped as to be lost amid small fluctuations of a more irregular sort.

The problem, then, is to determine whether some biological characteristic of the populations requires modification of the equations in such a way as to produce an extreme damping effect. Examination reveals no serious errors in equation (5) for the prey. In the paper cited, there was evidence of a continuous, rapid rate of growth of the diatoms throughout the flowering. Also the zooplankton appeared to filter at a relatively constant rate, irrespective of the quantity of phytoplankton in the water, and this has been substantiated by later work. Thus the populations approach the ideal specifications for b_1 and k_1 . However, the English Channel observations showed that the zooplankton utilized only a small part of the food that they ingested during the flowering period. The faecal pellets were green with discarded plant material. Food ingestion was estimated to be of the order of 50 per cent of the animals' weight per day; assimilation probably was no more than 10 or 15 per cent. During the remainder of the year, assimilation appeared to be more nearly complete. Thus the zooplankton did not fulfil the requirements of equation (6). The assimilation coefficient k_2 was not constant, but rather it varied inversely with N_1 . The expected results are (a) predation during the late stages of the flowering will be less severe than would be the case if all the food consumed were utilized more effectively to increase the zooplankton growth rate; (b) with lesser reduction of prey and more effective utilization of it after the flowering, the starvation and decline of predators will be less extreme. Qualitatively, these are sufficient reasons for a damping effect on the oscillations of both predators and prey. Quantitatively, it is probably only a partial explanation, since it fails to take into account various other ecological factors that may be important.

THE DEVELOPMENT OF QUANTITATIVE ECOLOGICAL THEORY

Fleming (1939) developed a simple mathematical model of phytoplankton and zooplankton grazing relations based on the English Channel observations described above. He postulated a differential equation for phytoplankton growth that was closely analogous to Volterra's treatment of prey. Observational data were used to evaluate a constant growth coefficient, synonymous with b_1 in equation (5). The grazing term was written in a different way, but with the basic meaning of a constant grazing coefficient (k_1) and a zooplankton population (N_2) that increased in a linear fashion, approximating the observed increase in nature. At the beginning of the flowering, the growth coefficient was larger than the grazing factor, but the latter increased with time until it was larger. Thus the phytoplankton population first increased and then decreased. The population curve—the integral form of the equation—was a bell-shaped, symmetrical curve that approximated observed spring flowerings.

The equation was framed upon assumptions that were admittedly oversimplified, but in general they agreed with the observations available at that time. The assumed linear increase of zooplankton, although arbitrary, was more realistic than to make the zooplankton growth rate strictly dependent upon the quantity of phytoplankton. In a later attempt to clarify the theory of zooplankton growth (Riley, 1947), it was assumed that growth during the flowering depends upon an arbitrary maximum rate of assimilation which cannot be surpassed. However, it was also assumed that Volterra's equation (6) for predators is applicable at any time when the available food supply is small enough for $k_2 N_1$ to be less than the maximum rate.

In one respect, Fleming's analysis was a radical departure from previous population studies. Instead of dealing with individual species, the whole phytoplankton association was lumped into one unit and the zooplankton into another. Although the concept of ecological groups has been used for many years in qualitative ecological discussions, it is distasteful to many biologists to take the further step of treating the ecological group quantitatively as a sort of super-species. However, we have neither the biological knowledge nor the mathematical ability to treat all the species individually. Whether the necessary simplification is justified can be judged only by the statistical validity of each result.

At the time Fleming's paper appeared, the writer was beginning a two-year survey of Georges Bank, an offshore area of shallow water marking the southern boundary of the Gulf of Maine. Routine observations of plankton and various environmental characteristics of the region were obtained. In addition, there were experimental estimates of the rates of photosynthesis and growth of the natural phytoplankton association. These were originally intended for the purpose of describing phytoplankton productivity. However, analysis of the results revealed good correlations between physiological processes and certain environmental factors, and this paved the way for a more extensive treatment of the ecology of phytoplankton seasonal cycles.

The analysis was developed (Riley, 1946) from a basic differential equation for phytoplankton growth that differed only in minor details from Volterra's

equation for prey. The equation was then expanded by considering each coefficient as an ecological variable rather than a constant. This seemed a logical way to attack the problem because we know that ecological factors operate by modifying individual physiological processes of the organism. In nature as well as in the equations, the growth rate is the net result of the operation of a series of such processes.

The Georges Bank experiments showed a linear relationship between solar radiation and the photosynthetic rate during the winter and spring. This supplied a coefficient that made it possible to estimate the average photosynthetic rate of the whole phytoplankton population at any time during the spring period, simply by estimating the average intensity of light in the growing zone. This involved a complex of factors—incident radiation, transparency of the water, and the depth of the so-called mixed layer (the surface layer of nearly homogeneous water). It was also necessary to introduce a nutrient factor, since insufficiency of phosphate was found to depress photosynthesis during the late spring, summer, and early autumn. The respiratory rate appeared to vary with temperature.

The difference between photosynthesis and respiration was assumed to equal the growth coefficient b_1 in equation (5). The grazing factor was compounded from a constant and the quantity of zooplankton, as in the expression $k_1 N_2$ of Volterra's equation. In short, the equation for phytoplankton was rewritten in terms of physiological constants and six environmental factors: solar radiation, transparency, depth of the mixed layer, phosphate, temperature, and zooplankton. The results are shown in Figure 2.

While the method of analysis obviously oversimplified the problem in many ways, the over-all results seemed realistic enough to warrant further investigations. Lillick (1937) had described a seasonal cycle in the coastal waters of southern New England, in which the spring flowering was early, and which differed in other ways from the Georges Bank population. The same equation was applied to these waters and also to a two-year series of observations on Hulan Harbor, Korea, described by Kokubo (1940). These provided a further test of the equation in that the plankton cycles and accompanying environmental characteristics differed considerably from one year to the next. The results, shown in Figure 2, demonstrate general agreement with observations. In other words, the local characteristics of the seasonal cycles appear to have been determined primarily by variations in the six environmental factors listed. The reasons for the differences have been discussed in the original papers (Riley, 1947a; Riley and Von Arx, 1949) and will not be dwelt upon here. It suffices to say that the changes in the phytoplankton population nearly always involve a subtle interaction of several factors; seldom does any one of them dominate the situation. It is a type of problem in which reasonable explanations are seldom obtained by qualitative logic. Quantitative analysis is required to elucidate the relative importance of different factors. The methods described, crude as they are, have provided some insight into these matters. Perhaps more important is the fact that the discrepancies between theoretical and observed populations have spotlighted problems that need further investigation.

Thus the phytoplankton analysis transformed the Volterra theory into a promising ecological tool; however, an important characteristic of the prey-predator equations was lost along the way. There is a mutual dependence between these two populations, which is expressed by the inclusion of an N_1 function in the N_2 equation and vice versa. The analytical solution is a simultaneous solution of the two equations. Ecologists recognize the existence of the same kind of mutual dependence in nature. Zooplankton growth depends upon the quantity of available phytoplankton food, but conversely, zooplankton grazing

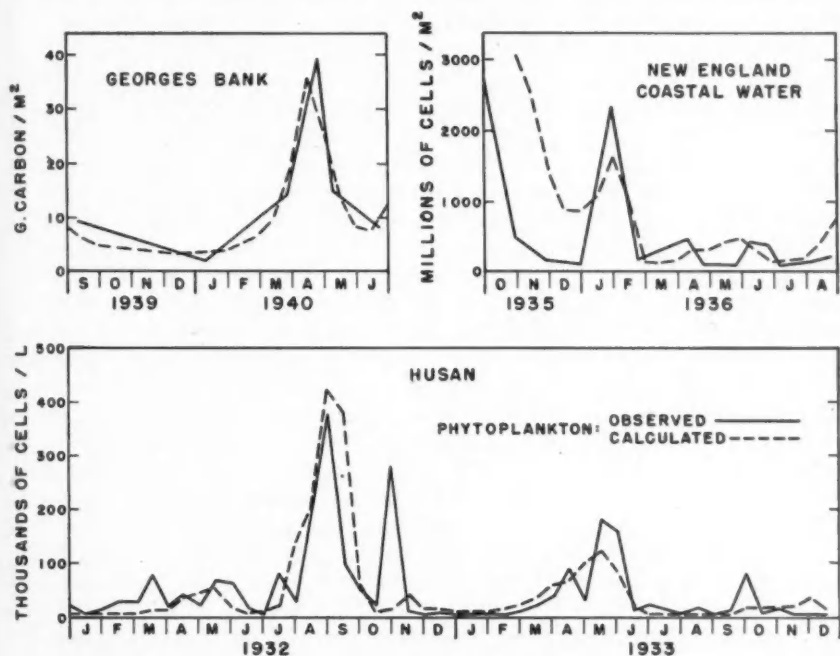


FIGURE 2. Comparison of observed seasonal cycles of phytoplankton in three areas with calculated seasonal changes as determined by application of a theoretical ecological equation. For further explanation see text.

influences the phytoplankton concentration. So on from one step in the food chain to the next. Also, each member of the animal and bacterial population releases inorganic nutrients which influence phytoplankton growth; conversely, the latter affects the nutrient concentration. The biological association as a whole—plants, animals, and their component chemical elements—form a vast, intricately related, and interdependent system. This concept was expressed in ideally simple terms by Volterra. It was abandoned by Fleming, who postulated an arbitrary rate of zooplankton growth, and by the writer, who used observational data on zooplankton and phosphate. These short cuts accomplished the immediate purpose of

describing seasonal changes in phytoplankton. They failed to elucidate the broader principles of ecological relations, and in some respects this was a serious deficiency.

Ordinary ecological reasoning supplies the principles for a broader quantitative theory. The main problem is to translate principles into mathematical terms, a process that has only just begun. Every environment possesses certain distinctive physical features—properties of geography and climate—that determine what kind of population can invade it, and which continue to exercise a control of the population by influencing the rates of all life processes. These are independent factors; they exist whether there is a population or not, although the way they operate depends upon the physiological behaviour of every organism in the community. In addition, the population brings with it a maze of interdependent biological relationships. The ecologist attacking this problem logically proceeds from environmental causes to biological effects. However, only the geographic and climatic factors are basically causal. A biological population is a cause of one kind of activity and the result of another. When cause and effect are completely intermixed, they must be regarded as the complicated result of the independent physical processes.

The phytoplankton equation previously described contained terms for light, temperature, and other reasonably valid physical factors. It also included phosphate and zooplankton, both of which are to a large degree biological properties. A full-scale treatment of the ecological system would require writing equations for phosphate, zooplankton, and other members of the Georges Bank association and solving all of the equations simultaneously.

Riley, Stommel, and Bumpus (1949) made preliminary tests of this type of analysis in five areas of the western North Atlantic, ranging from temperate coastal waters to an oceanic region in the subtropics. An abbreviated food chain was postulated consisting of phytoplankton, herbivorous zooplankton, their predators, and phosphate. Equations were written for each group, and the necessary physiological coefficients were inserted. At this point there were two major problems. First, the equations demand detailed, quantitative knowledge of a variety of growth processes. A review of physiological and ecological data revealed some of the necessary information; the remainder was little more than guesswork. Second, the distinction between basic regional factors and biological relationships, however sound it may be, does not always work out well in practice. For example, the soil on land and the deep-water concentration of nutrients in the ocean depend partly on what was there originally and partly on the action of biological agencies over a long period of time. A long-period study would include the history of their evolution. In a short-period investigation it is more practical to regard them as a fundamental part of the environment. Thus the deep-water concentration of phosphate was chosen as a primary environmental factor, together with radiation, temperature, vertical turbulence, and transparency. The last item was also somewhat objectionable, since the concentration of phytoplankton affects the transparency of the water. Eventually it should be regarded as a biological entity, but for the time being it was desirable to keep

the analysis as simple as possible. The problem was further simplified by assuming the existence of a steady state; in other words the calculation determines the quantity of each ecological group that can exist as an equilibrium population with any particular array of environmental factors.

The mathematical treatment consisted of equations for phytoplankton and phosphate at a series of some twenty depths and the mean concentration of herbivorous and carnivorous zooplankton in the vertical column. The aim is to obtain a simultaneous solution of all the equations. This is possible, since all of them are interrelated, either because of biological relationships or because of vertical exchanges of the population effected by vertical turbulence. The solution of the equations was not as difficult as might be supposed. Southwell (1946) described a so-called "relaxation" method which will obtain an approximate solution of any number of interrelated equations. It is an arithmetical procedure, simple but time-consuming.

The results obtained from the study were of the right order of magnitude. The large differences between observed populations in temperate and subtropical seas appeared in the mathematical models with errors of not more than 25 per cent on the average. However, minor details of distribution were not depicted as accurately as in earlier theoretical studies and this was hardly to be expected in view of the complexities of the analysis.

A further study of the subtropical plankton population of the Sargasso Sea is now in progress. The observational part of the program is a two-year series of plankton collections and data on weather, temperature, and transparency that are being obtained by U. S. Coast Guard weather ships. This is of considerable value from a descriptive standpoint, since our knowledge of seasonal cycles in midocean waters is meagre. In addition, an attempt will be made to refine the theory of the pelagic food chain and use it to calculate seasonal cycles of phytoplankton, zooplankton, phosphate, and transparency. The primary environmental factors will be the phosphate concentration in deep water and the seasonal cycles of radiation, temperature, and vertical eddy diffusion.

This work has not proceeded far enough to predict how realistic the results are likely to be. However, the pelagic oceanic communities have a relatively simple structure and are the logical proving ground for quantitative ecological theory. In coastal waters, on the other hand, there is a benthic community of amazing complexity and diversity. It generally bulks larger than the plankton and has a profound effect on the whole biological system. Life habits are specialized; they do not easily fall into generalized categories. Theoretical analysis of the coastal community is an awesome task. In the English Channel, in southern New England coastal waters, and various other places, oceanographic laboratories have established broad programs that are gradually accumulating information on the marine community as a whole. Perhaps such work will eventually lay the basis for the theoretical analysis of inshore waters, but at the moment descriptive studies are likely to be more valuable.

Nor is it likely that food-chain theory will have any immediate, direct application to fisheries problems, in the sense of calculating the quantity of any

particular species. However, indirect and semiquantitative applications, which are more easily obtained, may also be rewarding. Cooper (1948) and other members of the Marine Biological Laboratory at Plymouth, England, have demonstrated that long-period trends in the pelagic fishery are correlated with the winter supply of inorganic phosphate in the water. If the relationship between pelagic fisheries and basic productivity is as close as these results suggest, a practical application of quantitative plankton theory might be valuable.

The demersal fish, with their highly individual habits, are not easily fitted into the pattern of quantitative ecological analysis. The total quantity of demersal fish probably is related in an obscure way to the quantity of plankton that reaches the bottom, but a generalized criterion of this sort would be of slight value in determining the quantity of any one species. Perhaps theoretical methods will be applicable to feeding problems of the larvae of demersal fish. In the study of year-class fluctuations, feeding is presumably only one of a series of factors, but recent evidence suggests that the whole problem may not be as difficult as it seems. Carruthers (1951) has tackled the apparently tremendous problem of the effect of physical oceanographic processes on fish migration and larval dispersal. He discovered a very simple relationship. He was able to predict the recruitment of several commercial fish stocks from barometric-pressure gradients. Admittedly the operation of the relationship between wind and fish is complex. It probably encompasses a series of quite different phenomena. But the mere existence of this correlation is sufficient evidence that the complexities of the higher members of the food chain, which we often regard as too difficult to analyse quantitatively, sometimes cancel out and leave a precise causal relation.

Thus three investigations have been cited supporting the concept that the major effects in a biological population are controlled by a few key processes, regardless of how complicated the details may be. A mere handful of meteorological and oceanographic factors will determine the major features of seasonal and regional plankton variations. Long-period trends are accurately reflected through several steps in the food chain, from phosphate to herring and dogfish in the English Channel. The abundance of herring, haddock, and other North Sea fish stocks depends upon winds.

The goal of quantitative ecological theory is to reduce the environmental factors to what we know are the real fundamentals—meteorological data and geographical facts—and use them to determine both the biological history of a local water mass and the modifying effects of animal migration and physical oceanographic dispersal. In the investigations cited we begin to see some of the separate elements of this problem, waiting to be combined and developed.

Probably the results will never be all that we might hope for. They will give us only generalized information, and it will not be as accurate as direct observations. But ecologists are already swamped by the burden of routine observations, and every time we discover another important factor, the burden gets heavier. It is an attractive idea that we might get something for nothing, and theoretical analysis is practically free. It utilizes a simple sort of data that is, or can be, routinely collected by weather stations, weather ships, and fishing

vessels. The data can be processed inexpensively by automatic computers. Perhaps it is only by such short cuts that we can hope to obtain routine coverage of large areas and still have time for other investigations of a more specialized sort.

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The Standing Crop of Net Plankton in Lakes

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ABSTRACT

Plankton sampling has been a part of the program in a number of investigations of lakes in western Canada during the past 20 years. The techniques chosen for this work are discussed and criticized. The resulting data on average standing crop of net plankton are summarized and considered in relation to the kinds of lakes represented. The standing crop in 20 lakes shows an inverse relation to mean depth which is interpreted as indicating that, in most of these lakes, the trophic condition is greatly influenced by morphometry. Deviations from this relation appear to be explained by the secondary effects of climate and edaphic situation.

The difference in quantity between the standing crops of net plankton in oligotrophic and eutrophic lakes is not large, and, of course, these lake types intergrade. This lack of sharp differentiation is further obscured by rapid and extensive seasonal fluctuation in amounts of plankton and by the difficulties inherent in present methods of sampling. These circumstances render measurements of standing crop difficult and of only moderate utility in suggesting the trophic type or the possible productivity of a lake.

INTRODUCTION

THE AMOUNT of plankton in lake waters is of great interest both in theoretical and applied limnology. In the fundamental field we need to measure the quantity of plankton and its metabolic activities in order to work out the dynamics of productivity. Such knowledge, as it becomes available, will also be useful in the applied field. In the meantime we would like to measure at least the standing crop of plankton, to see whether it can be used in the classification of lakes and in the prediction or management of their fish production.

Studies of marine plankton have been going on for about one hundred years and in the past fifty years there has been much excellent work on fresh-water plankton. Investigations have shown the numbers and variety of plankton organisms and something of their geographic distribution. Continued studies have demonstrated extensive seasonal and annual variation in the quantity and composition of plankton, and we now know something of the vertical and horizontal distribution of plankton forms. All of this work has revealed a very complex community and one which is not easily measured or defined. The question is whether these complexities and variations are so great that nothing short of long and continuous investigation will give worth-while results. Can we devise direct and reasonably short procedures for sampling and measuring which will provide information usable in the problems of applied limnology and fisheries? It is the purpose of this paper to review the general problem, to report on a method of sampling used in lakes of western Canada and to discuss briefly the application of sampling results in lake ecology.

SOME GENERAL CONSIDERATIONS

Before embarking on quantitative studies of lake plankton it is necessary to consider what we mean by plankton, to recognize its complexity of composition and to decide what fraction of total plankton and associated materials is to be sampled.

WHAT IS PLANKTON?

The original definition of plankton by Hensen (1887) included all floating organic materials, dead or alive, as long as they belong to the organisms. It was thus nearly equivalent to the material which Kolkwitz (1912) termed "seston" and defined as all solids which can be strained from the water. In attempting to define and describe plankton, investigators have named about 60 kinds (Naumann, 1931) with reference to composition, size, mode of capture, distribution, origin and life-history. Most of these terms have been little used and for our purposes we may restrict our consideration to matters of size, living and dead elements and to distinguishing between truly planktonic materials and those which are of non-planktonic origin.

At the upper end of the size range a few organisms, such as *Mysis* and *Corethra* larvae present a problem which we may solve or evade, by saying that they are not typical plankters and removing them from the sample. At the lower end of the range we distinguish, in theory, nanoplankton which includes organisms smaller than 25 microns according to Birge and Juday (1922) or 60 microns according to Naumann (1931). I say, in theory, because fine mesh nets take some nanoplankters, and filters or settling techniques collect some of the larger forms. Thus in practice we cannot separate or measure the two groups satisfactorily. In this connection also, high-speed centrifuging collects the widest range of living and dead plankton organisms plus much organic and inorganic suspended matter (tripton), but it still fails to remove some of the bacteria and other very minute particles. These constitute the "ultraplankton" which Naumann (1931) defines as organisms less than 5 microns in length.

It is well known that the seston, or plankton as we continue to call it, contains considerable quantities of non-living material and that the proportion of this material in the plankton from a given lake will vary somewhat with the method of collection. Birge and Juday (1934) suggest that about one-half of the centrifuge plankton of Trout Lake was made up of colloidal and suspended organic material. In net plankton the non-living fraction is presumably smaller but we have no good way of determining it. Occasionally, as in heavily silted waters, inorganic material is abundant. Some correction can be made for this condition by determining the amounts of ash and organic matter. However, if we compare the ash content of diatoms with that of copepods, we see that the percentage of organic matter varies widely even in living plankton. Again, organic materials such as wood fibres or other allochthonous debris may occasionally contaminate the plankton to such an extent that organic determinations are of little value as an indication of the amount of living plankton present.

PLANKTON QUANTITY AND COMPOSITION

The standing crop of centrifuge plankton (seston) in Lake Mendota was found by Birge and Juday (1922) to range from 141 to 287 kg./ha. dry organic matter, with an average of 240 kg./ha. In the deep-water region (20 m.) the average was 435 kg./ha. Trout Lake in northeastern Wisconsin had an average of 128 kg./ha.

The relative amounts of plant and animal forms in the plankton can be approximated by Lohman's (1912) method of determining the unit volume of each species and using these values to convert numbers to volume. By this means, Ruttner (1940) finds the average phytoplankton:zooplankton ratio in alpine lakes in summer to be 1:2 but in some lakes as high as 1:15. Riley (1940) found a ratio of 1:0.22 in Linsley Pond, Connecticut. Birge and Juday (1922), by determining the weights of representative species, deduce that the zooplankton contributes one-third of the organic matter in the net plankton of Lake Mendota, giving a ratio of 1:0.5. Birge (1934) indicates that the zooplankton may make up 28 per cent of the centrifuge plankton of Trout Lake and suggests that about half of the centrifuge plankton may be non-living. Thus the p:z ratio might be about 1:1.2. After considering these data, it is rather surprising to find that Juday (1940) suggests that the zooplankters make up about 6 per cent of the total plankton of Lake Mendota and refers to the remaining 94 per cent as phytoplankton. Ruttner (1940) also has questioned the inclusion, by some American workers, of non-living tripton with the phytoplankton. While the data are scanty, it seems probable that in alpine and other oligotrophic lakes the zooplankton is often greatly in excess of the phytoplankton, while in eutrophic lakes the reverse is usually true.

The weight of net plankton (No. 20 silk) in Lake Mendota was one-fifth that of the centrifuge plankton. In Green Lake, Wisconsin it was one-twelfth. Juday (1922) suggests that the usual range is one-third to one-tenth. Using the average of one-fifth and knowing the percentage of ash in net plankton (23.5) and of centrifuge plankton (55), the values for organic matter cited above may be used to calculate the average dry weight of net plankton in Lake Mendota as 177 kg./ha. for the deep water and 97 kg./ha. for the whole lake.

The ash content of the net plankton in Lake Mendota ranged from 9 to 48 and averaged 23.5 per cent (Birge and Juday, 1922). In a large series of lakes in western Canada we have found ash content usually between 25 to 45 per cent with an average of about 33 per cent. In a few samples with many copepods and very few diatoms the ash content was between 15 and 20 per cent. The amount of diatoms in the plankton of our lakes appears to be the main cause of variation in the ash content. This effect is shown in another way in Ricker's (1938b) data for Cultus Lake where, in March with an abundance of diatoms, the total nitrogen content was only 3.5 per cent of the dry weight but in the summer plankton, with zooplankters dominant, it was 7.1 per cent. Muddiness or high silt content may also result in unusually high ash percentage. Such examples suggest the desirability of determining organic content as well as dry weight of plankton samples.

The dry weight of net plankton has been estimated as approximately 10 per cent of the live or wet weight by Birge and Juday (1922) and by Ruttner (1937). Tests made on net-plankton samples from Waskesiu Lake gave values of 8 to 14 per cent. There would seem to be no satisfactory way of determining when the extraction of water from a sample of wet plankton is complete or when it begins to reduce its normal quantity of body fluids. The same difficulty is encountered in the determination of plankton volumes by filtration or displacement methods.

Volume determinations of lake plankton by settling are very erratic, since some samples settle compactly while others remain loose and flocculent. Ruttner (1940) refers to settling volumes as worthless. Sheard (1947), after critical tests on marine plankton, concludes that settling is unreliable, and finds that displacement methods provide reasonably satisfactory determinations of volume.

STANDING CROP AND PRODUCTIVITY

The significant question is, how long does the crop "stand" or in other words how long does it take to produce new materials equal to the weight of plankton present at the beginning of the period under consideration? The rapid increase in phytoplankton in cultures, and the sudden development of pulses in lake plankton, suggest a rapid turnover. Birge and Juday (1922) suggest one to two weeks and Juday (1940), in his energy budget for Lake Mendota, uses two weeks as the year-round average, suggesting that the turnover would be faster than this in summer and slower in winter. Hutchinson (1941) suggests that this estimate is probably too low, i.e., a faster turnover. Although we have, as yet, little more than opinions on this problem it has been attacked in several ways by Hutchinson and his associates Lindemann, Riley and Deevey. The growing knowledge of the dynamics of production may soon provide us with good answers to the question of the rate of turnover.

In this connection we may quote Riley (1940), that in Linsley Pond he found no correlation between standing crop and gross production for the whole lake. (There was, however, a positive correlation between standing crop and production in the surface water.) This conclusion and the relatively small range between the standing crops of plankton in oligotrophic and eutrophic lakes (data presented below) give us little encouragement to hope that the measurement of standing crop will be of much use as an index of productivity.

SELECTION OF A SAMPLING TECHNIQUE

For the program of lake studies in western Canada it was necessary to choose sampling methods keeping in mind the kind of information desired, the time available and the above-mentioned general considerations as to kinds and qualities of plankton. The decision was made to work on the net plankton and to use No. 20 silk since that takes, in a single operation, both the zooplankters and a fair quantity of the phytoplankton species. As Ricker (1938a) points out, nets of No. 10 silk are more reliable for sampling most zooplankters. However, the use of No. 10 nets would seem justifiable only if they could be supplemented

by settling or centrifuge determinations of the nannoplankton. The great variability of the p:z ratio indicated by Ruttner (1940) suggests that a sampling method which takes mostly zooplankters may not provide a good measure of the total plankton crop.

Nets, 10- and 20-litre traps and a Clarke-Bumpus sampler have been used in the Canadian lakes on which we are reporting. The traps and sampler were used mainly for the calibration of the large closing net which Ricker (1938b) identifies as the Rawson net although it is essentially similar to the larger Wisconsin net of Birge and Juday. Our nets have a mouth diameter of 25 cm. and a truncated cone of heavy cotton 30 cm. long leading to a centre ring 30 cm. in diameter. The straining cone is 60 cm. long and carries the usual straining bucket, 5 cm. in diameter and with a bayonet attachment. These specifications have been rigidly maintained during the 20-year period of our investigations on western lakes. This net was adopted because, in vertical hauls, it took sufficient quantities of plankton for convenient determinations of dry weight, ash, and if desired, microkjeldahl determinations of total organic nitrogen.

The abundant warning provided by Ricker (1932, 1938a), has led us to intensify various precautions used to preserve the efficiency of these nets. New nets were used each summer and on each lake. They were washed and dried between periods of sampling and they were not used for surface tows. Nets were hauled at a uniform rate of 0.5 metres per second. One metre per second would have been a better choice, since recent tests have shown increased straining efficiency at speeds up to about 1.7 m. per sec. Calibration for straining efficiency was usually carried out at the beginning, in the middle and at the end of the season. The method used was to take 10-litre trap samples at each metre depth down to 10 m. and to compare the dry weight of the combined catch with that from a 10-metre vertical haul with the net to be tested. We find this method is quicker and less subject to error than the method of counting organisms in fractions of the hauls. A new type of 10-litre trap, consisting of a cylinder one-half metre in length and closing with damper-type valves at each end, has been found to operate more easily and with less leakage than the commonly used Juday trap.

Our calculated net efficiencies have varied from 25 to 55 per cent in spite of the precautions mentioned above. The three or more calibrations during the season usually, but not always, indicate a gradual decline in the efficiency. It is recognized also that the efficiency of a net decreases progressively as it is hauled up through the water, a situation neatly compensated for by the new pumping sampler of Langford and Fry. Clogging during the haul is not believed to have been a major factor in our work, since most of it has been on oligotrophic or mesotrophic lakes with a thin plankton and little blue-green or gelatinous coated algae to cause rapid clogging of the mesh. In any case we had little choice since much of the work was done in very deep lakes (e.g., Great Slave 600 m.) where extensive use of traps would have been impossible in the time available. In such situations, the Clarke-Bumpus sampler with a suitable windlass was found useful. For smaller and shallower lakes, especially those with a heavy phytoplankton, we would not recommend the use of silk nets for most kinds of quantitative

sampling. Surface-plankton samples have been taken by the simple method of dipping 50 litres of water and pouring it through a No. 20 net.

Decision as to the number and location of stations for plankton collections in a lake involves the problem of horizontal distribution. Ricker's (1938b) careful study in Cultus Lake indicates an essentially random distribution of zooplankters in the open water of that small (6.3 km.²), deep lake. He refers to data from European lakes which suggest that this may be fairly general in such lakes. Chandler (1940) believes the horizontal distribution of phytoplankton to be reasonably uniform in the "islands region" of western Lake Erie. However, Wright and Tidd (1933) and Verduin (1951) have shown that when the whole west end of the Lake Erie is sampled, there are wide horizontal variations in the quantity of both phyto- and zooplankton. Langford (1938) demonstrated that the horizontal distribution of entomostraca in Lake Nipissing was far from uniform or random even at stations within a radius of three-quarters of a mile.

Where a lake basin is irregular in outline or in bottom contour, physical and chemical differences are readily observed in different parts of the lake. It is to be expected that the plankton crop will also differ in such areas. Observations in Great Slave Lake, N.W.T., and in Lac la Ronge, Sask., have demonstrated this situation. Therefore, our practice has been to establish stations for the collection of plankton in locations which appear to be representative of the main regions of the lake. In large and irregular lakes, the limitations of time often make it impossible to use as many stations as would seem to be desirable.

Seasonal changes in the quantity and quality of plankton are so extensive and so rapid that frequent and extended sampling at each station is obviously desirable. Data from alpine lakes of the Canadian Rockies (Rawson, 1942) and recent studies on Great Slave and Lac la Ronge suggest that sampling at weekly or ten-day intervals is usually satisfactory for following the major changes in the plankton crop. This sampling should, of course, be carried on throughout the major part of the growing season.

METHODS OF ANALYSIS AND EXPRESSION OF RESULTS

The simplest method of measurement, that is volume determination, has been mentioned above as of little value. In one special circumstance we have found it useful. When calibrating nets in the field by the method described above, volume determinations by settling or centrifuging provide a quick method of determining efficiency. Since both samples are of the same composition and of roughly similar volumes, the degree of settling is comparable. Later dry-weight determinations are made in the laboratory to confirm the result.

Dry-weight determinations have been made by drying the plankton in porcelain crucibles in an oven at 60° C. for 48 hours or until a constant weight is reached. If the samples are preserved in formalin and lake water (containing minerals) some addition to the dry weight will result from the solution introduced when transferring the sample to the crucible. If the dry weight of plankton is less than 100 mg., we find it desirable to correct for this error by washing the sample with distilled water or by drying and weighing a volume of the preserva-

tive fluid equal to that covering the plankton. The chief deficiency of dry weight as an indication of the amount of plankton is its failure to take account of the variable quantities of mineral substances such as suspended silt or the silica of diatoms. Thus determinations of organic content are most desirable.

The organic content of our plankton samples has been measured by the rough procedure of ashing or by microkjeldahl determinations for total organic nitrogen. In recent years we have used only ashing, in the opinion that its accuracy is at least as good as the sampling technique, and because the ease with which it can be done makes it possible to take and analyse more samples than could be handled for microkjeldahls. As indicated above, all methods of determination of organic content are deficient in that we cannot distinguish between true plankton substance and allochthonous organic material which, in some samples, forms a considerable fraction of the whole.

Numerical analysis of samples has been used in some cases for calibration and in others for investigating vertical and horizontal distribution of species. The procedure is so time-consuming and the numerical records of total population are so difficult to interpret, that enumeration would seem to have little place in our general lake studies. Our procedure, however, has been to take duplicate pairs of total vertical hauls or trap samples at each station. Thus one sample is available for dry-weight and organic analysis while the second can be used for microscopic study of the dominant species or for complete counting if desired.

The expression of results in quantities of plankton per unit volume seems to us to be generally less useful than quantities per unit area. Amounts of plankton per unit volume are of course necessary in dealing with vertical distribution and migration of plankton forms. As an expression of the total standing crop, the average amount per unit volume may be misleading if the sample is taken in water much shallower or deeper than the mean depth of the lake. An attempt to deal with this problem has been made by McMahon (1948) who postulates the "effective depth" of lakes as that depth above which is found 80 per cent of the plankton in a vertical column. It has been our practice to establish in any lake at least three stations, one at approximately the mean depth of the lake, one in shallower and one near the deepest water.

PLANKTON CROPS IN LAKES OF WESTERN CANADA

The available data on standing crop of net plankton in lakes of western Canada has been brought together in Table I. The areas and mean depths of the lakes are included and the average crops of net plankton are expressed in dry weight and organic matter per unit area. Since number of lakes is not large and the number of samples from some of them is very limited, these data cannot be regarded as norms, but the general range is considered indicative of the magnitude of plankton crops to be expected in lakes of this area. As a well-established datum, there are included the average crops of net plankton in Lake Mendota and Trout Lake, Wisconsin. These values were obtained by Birge and Juday (1922, 1934) from many samples and through several seasons.

The lowest range of standing crops of plankton, from 10 to 20 kg. dry weight per ha., is found in some alpine lakes from the Canadian Rockies and the extremely deep oligotrophic basins, McLeod and Christie Bays in the east arm of Great Slave Lake. The average crop in several other oligotrophic lakes lies between 20 to 40 kg./ha. Several moderately eutrophic or mesotrophic lakes have

TABLE I. The average standing crops of net plankton (No. 20) in summer in 18 lakes of western Canada and two in Wisconsin.

	Area	Mean depth	Number of samples	Av. dry weight	Av. ash	Av. organic matter
	sq. km.	m.		kg./ha.	%	kg./ha.
British Columbia						
Okanagan	370	69.5	11	26.0	30	14.4
Paul	3.9	34.2	7	50.2	16	42.3
Cultus*	6.3	32	8	30.4
Alberta						
Bow	3.6	17.6	10	11.9	33	8.0
Waterton	9.6	69.2	13	19.2	30	13.4
Maligne	21.8	40.5	12	20.6	38	12.8
Minnewanka	13.0	38.1	12	32.0	20	25.6
Pyramid	1.3	8.7	39	23.6	18	19.4
Saskatchewan						
Waskesiu	70	11.1	16	90.5	37	56.5
La Ronge	1,300	12.7	111	64.3	36	41.2
Hunter Bay	130	20.7	27	32.0	20	25.6
Amisk	376	13.2	72	30.3	29	21.4
Nesslin	6.0	13.2	17	59.0	38	36.6
Last Mountain	230	7.6	48	158.0	39	96.7
Athabaska	7,900	26	19	36.5	49	18.6
N.W. Territories						
Great Slave (main)	19,400	41	88	20.3	38	12.6
McLeod Bay	1,590	120	8	9.0	26	6.7
Christie Bay	1,510	249	24	10.6	25	8.0
Wisconsin						
Mendota†	39.4	12.1	415	177	22.4	137.3
Trout†	15.8	13.8	many	94

*From Ricker 1938b, average of 8 summer samples. †From Birge and Juday, 1922 and 1934.

average crops between 50 and 100 kg./ha. Last Mountain, a eutrophic lake in southern Saskatchewan, shows rather wide variation from year to year, but its average crop to date has been 158 kg./ha. Lake Mendota, a strongly eutrophic lake well south of our range, had an average of 177 kg./ha. It is unfortunate that we do not have comparable data from any small eutrophic lakes in this region. Smaller eutrophic lakes may well have standing crops as great as that of Mendota, but probably not much greater, for the shallowness reduces the trophogenic volume, and heavy phytoplankton growth may reduce light penetration and thus lessen productive efficiency.

We have no mechanical means of classifying lakes into degrees of eutrophy and oligotrophy. However, in these large lakes the morphometric factors are important and the mean depth may thus provide a crude scale or index. Thus, in

Figure 1 the average standing crop of net plankton is plotted against the mean depth of the lakes listed in Table I. Analysis of regression has produced the curve and formula, $p = \frac{3765.0}{d^{1.5337}} + 8.0$, where p is the dry weight of plankton in kg./ha. and d , the mean depth in metres. In its logarithmic form this equation is $\log(p-8.0) = 3.5758 - 1.5337(\log x)$. The constant, 8.0, was determined by inspection and it suggests that a standing crop of plankton of the order of 8.0 kg./ha. may be expected in lakes of very great mean depth.

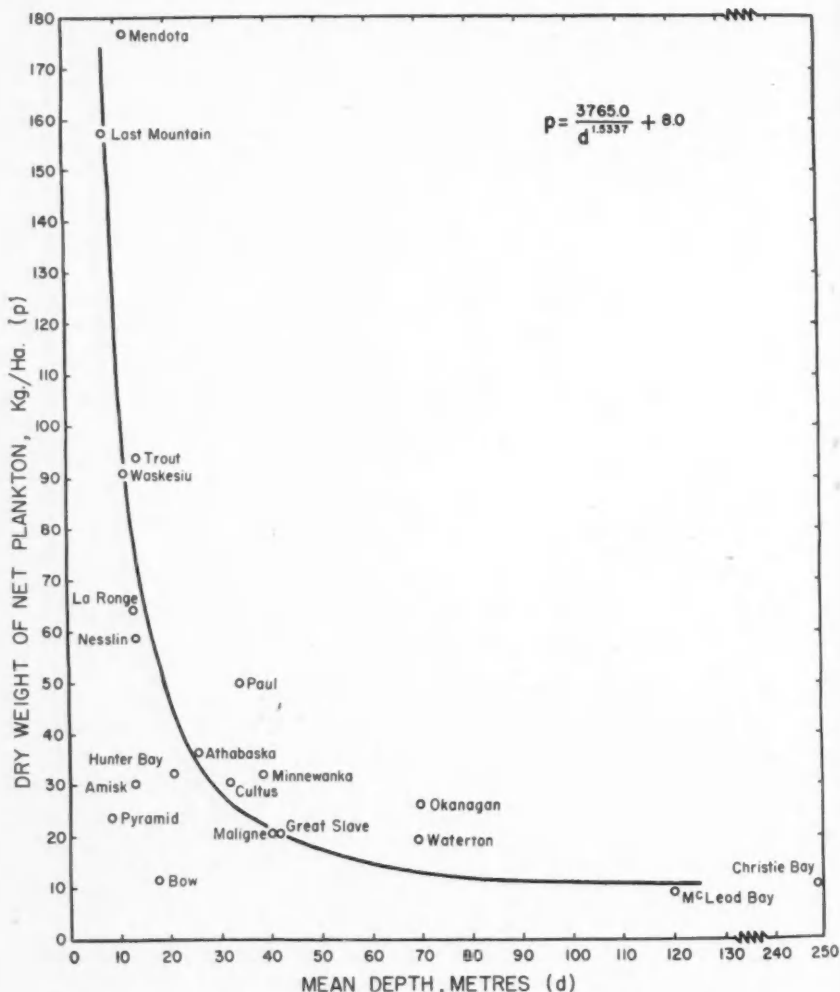


FIGURE 1. Mean depth and average standing crop of net plankton in 20 lakes.

While the points representing average plankton crop in most of the 20 lakes lie close to the curve, the values for six lakes show considerable divergence. Three of these, Paul, Okanagan and Mendota exceed the expected value and three others, Bow, Pyramid and Amisk, fall well below the curve. Paul Lake, although of depth which would usually produce oligotrophy, has been interpreted (Rawson, 1942) as having favourable climatic and edaphic conditions which have allowed it to develop eutrophic amounts of plankton and bottom fauna. Okanagan Lake also has favourable edaphic and climatic conditions. The standing crop of plankton in Lake Mendota is also very much greater than would be suggested by our equation. A part of this difference might be attributed to its location, much farther south and in a warmer climate than any of the other lakes under consideration.

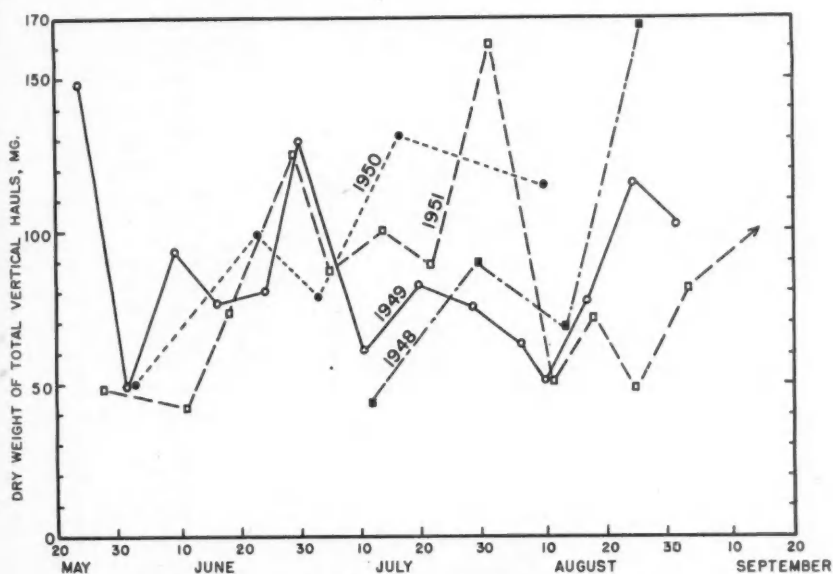


FIGURE 2. Seasonal variation in the plankton crop at a station in Lac la Ronge during the summers 1948 to 1951.

Of the three lakes in which the plankton crop is much below that indicated by the equation, Bow is the most divergent. This lake was noted by the writer (Rawson, 1942) as having extremely unfavourable climatic and edaphic situations which completely overshadow the effect of its shallow basin. Pyramid Lake (Rawson and Elsey, 1950) is also alpine and relatively cold, but its water is clear, in contrast to the heavy silt of Bow Lake, and it might thus be expected to produce more plankton than Bow. Amisk Lake, in northeastern Saskatchewan, is on the Precambrian Shield, has a low mineral content (Rawson, 1951) and may have low plankton production for both edaphic and climatic reasons.

McLeod and Christie Bays, with mean depths of 120 and 249 metres respectively, provide some indication of the minimum plankton crop in lakes of extreme depth which also have cold climate and low mineral content. These "bays" are great isolated bodies of water at the end of the east arm of Great Slave Lake. It will be noted that Christie, although twice as deep as McLeod Bay, has a slightly heavier plankton crop. This may be an edaphic effect since McLeod Bay has only one-fifth the mineral content of Christie (Rawson, 1951). The data suggest that in lakes deeper than 100 metres mean depth, increase in depth will have little effect in decreasing the plankton crop. This is recognized in the curve and equation by the use of the above-mentioned constant, 8.0.

The average quantity of net plankton (No. 10 mesh) observed by McMahon (1948) in ten lakes of the Skeena River drainage, British Columbia, was found to show a relationship to mean depth similar to that indicated in Figure 1. Three of these lakes with mean depths from 50 to 100 metres had amounts of plankton one-third as great as that of two lakes with mean depths of 7 and 10 metres.

The form of the curve in Figure 1 is suggestive of that relating average crop of bottom fauna to depth and area (Rawson, 1932, and reprinted in Rawson, 1939a). It is also similar to a curve showing the relationship between mean depth and long-term fish production in the Great Lakes and other large lakes in Western Canada (Rawson, 1952). It would appear that, in lakes of considerable size, morphometric factors are often dominant in determining the trophic condition and productivity.

SEASONAL VARIATION

Seasonal fluctuation is perhaps the greatest obstacle to measurement and interpretation of the plankton crop. Variations in the dry weight of plankton at a station in Lac la Ronge during the summers 1948 to 1951 are shown in the graph Figure 2. Samples were taken at intervals of one week to ten days. The amounts of plankton show the usual irregular fluctuations from week to week, superimposed on certain trends and marked by a few violent pulses. The calculated averages in the three years do not differ greatly, and the threefold range of variation (roughly 50 to 150 mg.) during the season is not excessive. However another station, among the islands in the northern part of Lac la Ronge, had an average standing crop during the same three years which was almost double that at the station represented in Figure 2. A comparable graph was used to show seasonal changes in the summer plankton of alpine lakes in the Canadian Rockies (Rawson, 1942).

Year-round records of total net plankton are provided for Lake Mendota by Birge and Juday (1922) and for Cultus Lake by Ricker (1938b). In Lake Mendota, spring and fall maxima are pronounced and separated by summer and winter minima in which the standing crop is about one-fifth of the maximum. It should be noted that, in some years at least, the spring maximum showed only in the nanoplankton and not in the net plankton. In Cultus Lake in 1932, Ricker found a prolonged maximum in spring and early summer, with a crop three or four times as great as that of autumn or midwinter. Summer records for Great

Slave Lake suggest poorly marked early and late maxima with a three- or four-fold range of variation in quantity.

Since, in the lakes cited above, the summer crop showed two- to fivefold fluctuations in quantity with no discernible pattern, it is evident that little reliance can be placed on measurements which do not cover a period of at least three months. There is also a possibility that the three-month average may differ considerably from year to year.

THE INTERPRETATION OF STANDING CROP

The range in average standing crops of net plankton observed in lakes of western Canada has been indicated in Table I as roughly 10 to 100 kg./ha., dry weight. In general the oligotrophic lakes had 10 to 40 kg./ha. and the moderately eutrophic lakes had more than double this. If, however, the standing crop of plankton is subject to a three- to fivefold variation during the summer season, it will require careful and extended sampling to distinguish lake types on this basis. It would seem to be too much to hope that, within this narrow range, the differences in standing crops of plankton could be related to basic productivity. It is suggested, rather, that the standing crop should be regarded mostly as a balance on hand which may not bear any close relation to the rate of turnover. There are also some theoretical grounds for suspecting a lack of relation between phytoplankton density and efficiency of production. The thin plankton of oligotrophic lakes with a deep trophogenic layer is undoubtedly more efficient than the dense phytoplankton which, in the surface layers of eutrophic lakes, impedes light penetration and thus restricts the trophogenic zone. The plankton of eutrophic lakes may be thought of as rich and lazy, that of oligotrophic as lean and hard-working.

QUALITATIVE DIFFERENCES OR TYPES OF PLANKTON

If the range of quantity of plankton in various lakes is too restricted to be of much use in classification, we may turn for help to qualitative differences. In the extensive literature on this subject, numerous plankton types have been distinguished by the phytoplankton species present. No one seems to have suggested why zooplankton species should not be equally distinctive.* Naumann (1931) lists ten such types recognized by dominance of families, (e.g., Chlorophyceae), genera (e.g., *Pediastrum*) and even species (e.g., *Fragilaria crotonensis*). Five of these types are listed as occurring in eutrophic and the other five in oligotrophic lakes. Pearsall (1932) after extensive studies on the English Lakes, suggests chemical explanations for abundance or scarcity of several phytoplankton groups. Hutchinson (1941) has modified some of Naumann's types, and adds the suggestion that the desmid type of plankton is found only in those oligotrophic lakes which are also poor in calcium. In a later publication Hutchinson (1944) dis-

*As this paper goes to press the writer has received an important contribution in this field. Jarnefelt has provided a detailed analysis of the occurrence of phytoplankton and zooplankton species in lakes of different trophic types: JARNEFELT, H. Plankton als Indikator der Trophiegruppen der seen. *Annal. Acad. Scient. Fennicae*, Ser. A. IV Biologica, 1-27, 1952.

agrees with some of Pearsall's generalizations concerning the chemical causes of phytoplankton periodicity. We thus have some basis for the qualitative distinction between oligotrophic and eutrophic plankton types, but the scheme is by no means certain or complete.

Various difficulties are encountered when we attempt to classify a lake from its phytoplankton species. Temporary fluctuation or succession may mask the basic type, and the occurrence of single species in profusion may still be misleading. Ruttner (1940), points out that *Asterionella formosa*, regarded as characteristic of cold-water lakes, appears to have a physiological race which tolerates warm water. In our large oligotrophic lakes of western Canada, *Melosira* is often dominant, although this genus is listed by Naumann as marking a eutrophic type. Thus, at the present time the recognition of plankton types on a basis of phytoplankton dominance is uncertain, and it would seem to require the knowledge of a specialist in this field.

CONCLUSIONS

1. Standing crops of plankton are difficult to measure satisfactorily. Among the main problems are the inadequacy of samplers and the existence of horizontal, vertical and seasonal variation in plankters. It is practically impossible to collect pure plankton or to separate it effectively from organic and inorganic contaminants.

2. The average summer standing crop of No. 20 net plankton sampled by total vertical net hauls in lakes of western Canada shows some distinction between oligotrophic and eutrophic types. The general range for alpine and large oligotrophic lakes appears to be from 10 to 40 kg./ha. dry weight. Mesotrophic and moderately eutrophic lakes have up to 100 kg./ha. Mendota, a strongly eutrophic lake, had 177 kg./ha. dry weight of net plankton.

3. The inverse relationship between standing crop of plankton and mean depth suggests that morphometric factors are predominant in determining the trophic type of most of the lakes under consideration.

4. The maximum quantity of plankton present during the summer is often three to five times as great as the minimum. Such variations make classification difficult, and suggest that the plankton present at any time may be regarded as a "balance on hand" during a rapid turnover and as such may have little relation to real productivity.

5. It is suggested that little reliance should be placed on the small number of plankton samples commonly taken in lake surveys. Qualitative plankton types require further clarification, but may prove helpful in recognizing trophic conditions.

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Methods of Plankton Collection and a Description of a New Sampler

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ABSTRACT

A discussion of the composition and distribution of plankton organisms forms a basis for the presentation of a critical review of methods of plankton collection. The quantitative sampling of plankton by pumps, water samplers, tow nets, plankton traps and the Clarke-Bumpus sampler is discussed. Data of a comparative nature dealing with some of the more usual methods are presented. A new instrument, the Toronto Sampler, developed at the Ontario Fisheries Research Laboratory, is described and its performance is assessed.

INTRODUCTION

THE QUANTITATIVE ASSESSMENT of plankton populations has been an important phase of the work of aquatic biologists ever since the original work of Hensen who published the results of his quantitative studies in 1887. A large number of methods of collection and analysis have been reported in the literature since that time. Many of these have been directed toward improving the efficiency of collection and minimizing the error in determining the quantity of plankton in the collected samples. A voluminous literature also exists descriptive of particular lacustrine or oceanic populations, their composition and their seasonal and spatial distributions, all of which must be considered when developing a method of sampling them quantitatively. It is the purpose here to review the problem of collection as related to our knowledge of the composition and distribution of plankton populations, and to describe a new sampler being developed by the Ontario Fisheries Research Laboratory. In addition certain data of a comparative nature dealing with some of the more usual methods of collection are presented.

COMPOSITION OF PLANKTON

Although Hensen, who introduced the term plankton, included detritus as well as organisms in that category, it seems preferable to separate the two and consider the organic and inorganic detritus *tripton* and the organisms *plankton* as two divisions of the *seston*. An acceptable definition of the term plankton is, therefore, that assemblage of organisms free-floating in the water independent of shore and bottom, and it is in this sense that plankton will be considered here. It should be borne in mind at the same time that almost all methods of collection sample both tripton and plankton and that the former may be relatively abundant at times. It is true also that in most gravimetric and chemical analyses no differentiation is made between the two.

Plankton has also been subdivided into many classes dependent upon the size, type, origin, environment and life-history of the organisms. Size classification is of prime importance, for the selection of the sampling method depends on the size range of organisms to be investigated.

On the basis of size, plankton is arbitrarily and somewhat unsatisfactorily divided into net plankton and nannoplankton. This was brought about by the work of Lohman (1908) who was the first to point out the extensive loss of organisms from the finest silk nets. The net plankton, considered as that portion retained in straining with a No. 20 bolting silk, includes the larger zooplankton and phytoplankton forms down to a minimum size of some 25 microns (Birge and Juday, 1922). Nannoplankton or the portion passing through such silk consists of organisms smaller than this. The division is arbitrary because, due to form or shape, a varying percentage of certain species may pass through the net and under certain circumstances some organisms that normally pass through such silk may be retained in rather high proportion.

SPATIAL DISTRIBUTION

It has been pointed out by Ricker (1937) that estimates of a plankton population made from total counts of individual samples are subject to statistical sampling errors. These can be determined and limits of confidence can be derived statistically. Such limits are valid only when the distribution of plankton is known to be random or when methods of collection are used which are designed to overcome the lack of randomness. In such instances only major variations, consistent differences over a period of time, or trends, will have much meaning. Hence a knowledge of the type of distribution, vertically and horizontally in a lake, is of utmost importance in quantitative measurements.

The marked differences in numbers of zooplankton organisms at different depths is so well known as to require no discussion. The existence of horizontal variations in distribution is, on the other hand, not as well recognized by limnologists. Although many investigators have assumed that plankton populations are randomly distributed horizontally, such randomness has been demonstrated in but a few instances. Ricker (1938a) found that the zooplankton of Cultus Lake, British Columbia, showed random distribution and, referring to data from certain European lakes, he suggested that this may be the case fairly generally in small deep lakes. Other workers have shown marked irregularities horizontally for both phytoplankton and zooplankton. Among these, Wright and Tidd (1933) and Verduin (1951) showed wide fluctuations in both groups in western Lake Erie. Moberg (1918) found greater than two-fold variations in numbers of zooplankton the rule in laterally spaced stations in Devil's Lake. The present author (1938) showed *Cyclops*, *Diaptomus*, nauplii and *Daphnia* to be bunched in their horizontal distribution at almost all depths sampled in Lake Nipissing. Essentially similar results obtained on the smaller lakes in Algonquin Park since that time lead us to believe that lack of randomness is common in zooplankton populations.

It would therefore appear that a lack of randomness should be assumed, in individual instances, until the opposite has been proven. Under such circum-

stances it is essential that this, together with vertical differences in abundance, must be taken into consideration in selecting sampling techniques.

SEASONAL DISTRIBUTION

That different genera and species of both phytoplankton and zooplankton rise to maximum abundance at different times and that the plankton as a whole varies with the season are common knowledge. In order that these changes in abundance may be taken into consideration, sampling intervals should be as short as practicable and sampling should extend throughout as much of the season as possible.

METHODS OF COLLECTION

There are a number of methods of sampling plankton organisms which have had more or less widespread application in limnology. These fall into two main categories: (1) the collection of water from which the organisms are separated by other equipment, and (2) the collection and separation of the organisms from the water in one operation. In the first class are pumps and water samplers generally. The second may be taken to include tow nets, plankton traps, the Clarke-Bumpus sampler and the Toronto sampler.

COLLECTION FOR LATER SEPARATION

PUMPS

Pump methods of collection have been used for many years. Hensen (1887) suggested the use of "tube and pump" for collection, and Kofoed (1897) indicates his use of pump collections in testing the efficiency of tow nets. Among many other investigations, Birge and Juday (1922) used pump methods and give a detailed description of the pumping and straining equipment used in their quantitative survey of the inland lakes of Wisconsin.

Throughout the years there have been a number of references to the fact that certain organisms seem to be sampled more effectively than others by pump methods. An explanation of these discrepancies that has been repeatedly given is that the currents produced in the water at the intake end of the hose result in rheotaxes by certain more active zooplankters. Although it is difficult to understand how an organism at some depth in a lake could detect such a current unless it possesses a sensory mechanism sensitive to acceleration or turbulence, nevertheless it is apparent that, in comparison with other methods, the collection of certain organisms is favoured by the pump, whereas other genera are taken less efficiently by pump and hose.

This is exemplified by very comprehensive data furnished the author by Dr. C. Juday of the University of Wisconsin some years ago. Since these data have not been published it is felt that the information gained from their analysis would be of interest and value in the present discussion.

Plankton samples were taken by means of a "semi-rotary" hand pump equipped with half-inch garden hose and pumping about five litres a minute, with a power pump of the Vane type pumping about 30 litres a minute through a one-

inch hose and with a 45-litre plankton trap. This equipment has been described by Juday (1916). Ten litres of water were pumped in each pump catch. No. 20 bolting cloth was used for all sampling, and the catches at a particular depth were taken simultaneously by the three methods. Twenty-nine separate comparisons were made of these three methods of collection. Triplicate samples were obtained by each method in each instance. The average numbers per litre of particular organisms obtained in the triplicate catches are compared by expressing the number captured by the pump as a percentage of those taken by the trap. Organisms which averaged fewer than 1.5 per litre are not considered.

TABLE I. Mean percentage efficiency of power pump compared to Juday trap.

	<i>Diaptomus</i>	<i>Cyclops</i>	Nauplii	<i>Daphnia</i>	<i>Diaphanosoma</i>
Mean	71.6	116.8	126.5	74.7	76.6
Variance s^2	126.2	622.5	339.4	952.1	473.1
Number samples	19×2	28×2	29×2	18×2	11×2
t	7.8	2.5	5.49	2.49	2.52
Probability	0.001	0.02	0.001	0.02	0.02

TABLE II. Mean percentage efficiency of hand pump compared to Juday trap.

	<i>Diaptomus</i>	<i>Cyclops</i>	Nauplii	<i>Daphnia</i>	<i>Diaphanosoma</i>
Mean	31.1	104.3	130.9	79.3	26.2
Variance s^2	182.1	670.6	724.8	1021.6	171.8
Number samples	19×2	28×2	28×2	17×2	11×2
t	15.7	0.64	4.3	1.88	13.1
Probability	0.001	0.5	0.001	0.06	0.001

Tables I and II show, for five organisms, the mean percentage efficiencies of power pump and hand pump when compared with trap collections. *Cyclops* and nauplii were taken in significantly greater numbers by the power pump than by the trap, and *Diaptomus*, *Diaphanosoma* and *Daphnia* were taken in significantly smaller numbers by this method. The hand pump appears to be about as efficient as the trap in capturing *Cyclops* but quite inefficient in sampling *Diaptomus* and *Diaphanosoma*. *Daphnia* too are captured rather inefficiently by the hand pump but nauplii are again taken in greater numbers by this pump method than by the trap. These results are similar to those reported by Birge and Juday (1922) who state "several sets of experiments made with the plankton trap and the power pumps showed an advantage in favour of the trap of 16 per cent in *Diaptomus* and of 15 per cent in the *Daphnia*, on the other hand there was a numerical advantage in favour of the power pumps amounting to 12 per cent in *Cyclops* and 22 per cent in the nauplii."

When the two pump methods are compared it is evident that their efficiencies are similar for *Cyclops*, nauplii and *Daphnia*, but that the power pump is more effective in capturing *Diaptomus* and *Diaphanosoma*.

WATER SAMPLERS

The collection of water by means of sampling bottles of various types has been rather generally used in plankton sampling, particularly in the investigation

of nanoplankton populations. The later separation of the organisms from the water has been carried out with membrane filters, hard-surfaced filter papers, sand filters or by means of centrifuging. These methods are described in some detail by Welch (1948). The method of sedimentation and counting with an inverted microscope as described by Utermöhl (1931) is now widely used as well. The small amount of water sampled by these procedures usually precludes the possibility of sampling the larger and scarcer forms at all accurately, although this does not seriously invalidate their use in nanoplankton studies. There is also the possibility that more active zooplankters may avoid such sampling instruments. Rawson (personal communication) has recently developed a closing-tube sampler, and one of somewhat similar design is being tested at the Ontario Fisheries Research Laboratory in Algonquin Park. It is thought that such a sampler will be of value when used to supplement the Toronto trap collections. The Toronto sampler, described later in this paper, is designed for use in deeper water, whereas the tube sampler, which has a tube length of from one-half to two metres may be more valuable for sampling in the littoral zone.

SIMULTANEOUS COLLECTION AND SEPARATION

TOW NETS

The tow-net method was the original sampling technique developed by Hensen. A conical net of silk bolting cloth may be towed through the water, thereby straining the organisms from the water which passes through the net. Since the net offers resistance to the passage of water, only part of the column is strained. Hence for quantitative results it is necessary to calibrate the net with respect to its straining capacity. In order to increase the efficiency of the net, a truncated cone of cotton or silk was attached to the open end (Juday, 1916), thus decreasing the area of the column strained with respect to the straining area of the net. In addition to taking total vertical hauls, such nets are used to obtain stage hauls since they can be closed at any desired depth.

Even with increased efficiency, the tow net is subject to criticisms, particularly if constructed of fine-mesh silk such as No. 20 bolting cloth. The efficiency may be fairly constant for a standard length and rate of haul with a particular concentration of organisms, but the main disadvantage lies in the fact that the efficiency does not remain constant in different lengths of haul and in different concentrations of plankton. Moreover the efficiency of such nets has been shown to change with age and the accompanying increased clogging, shrinkage and fraying of the threads.

Even as early as 1897, Kofoid pointed out some of these errors of the tow-net method and showed, by comparison with pump collection, that depending on the concentration of plankton organisms the coefficient of the net could vary from 1.5 to as high as 5.7. This demonstrated the impossibility of establishing a fixed coefficient of efficiency. He also showed that a fully shrunken net had the aggregate area of its openings reduced to less than 50 per cent of a new one. Ricker (1932) in comparing a new net, which had been soaked and dried several times, with one which had been used for one summer, found that the new net was

2.8 times as efficient as the old. In determining the efficiency of particular nets as compared with pump catches, he gives a coefficient of 5.6 for a new net and 14.4 for an old net, 18 per cent and 7 per cent efficient respectively. Other workers, MacKay (1924), Rawson (1930), Juday (1932), give a variety of coefficients most of which indicate better than 50 per cent efficiency. In most of these cases the calibrations were made with short hauls with the net, which may explain the high efficiencies.

Some comparisons between a new Juday net and the Juday plankton trap made by the author a number of years ago, showed wide fluctuations in the efficiency of the tow net in different lakes and in different concentrations of plankton. One graph, Figure 1, will suffice to indicate the extent of these variations. In two and a half-metre hauls near surface, the net was from 36 to 65 per

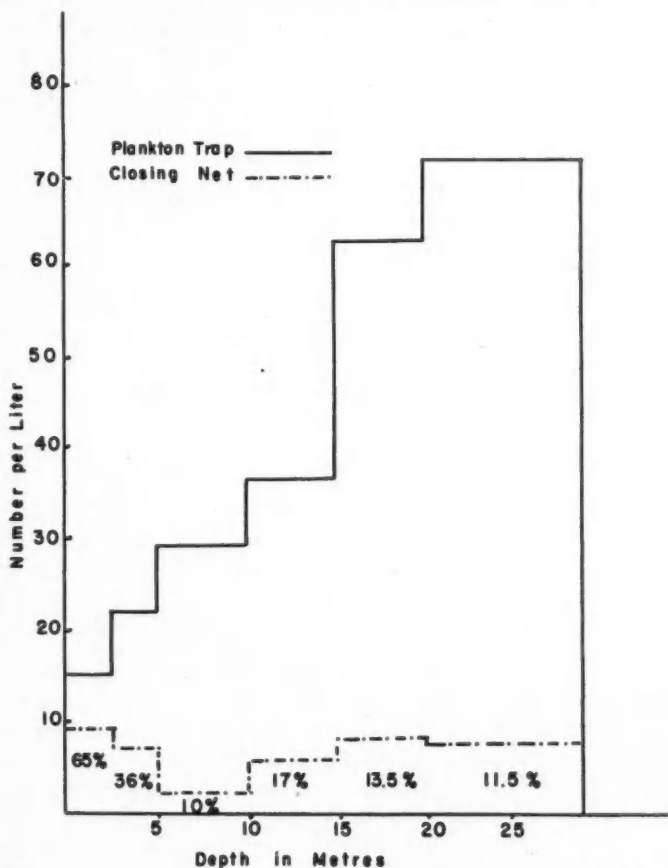


FIGURE 1. Comparison of closing plankton net and Juday trap in collecting total zooplankton organisms, Trout Lake, Wisc., Sept. 2, 1933. Net catches expressed as a percentage of the average of trap samples taken in similar strata.

cent efficient compared with the trap in capturing total net zooplankton. In five-metre hauls at lower levels it was only 10 to 17 per cent efficient and in a nine-metre haul at the deepest level where zooplankton was most abundant it was 11.5 per cent efficient.

Rawson designed a large closing net of No. 20 silk (dimensions given by Ricker, 1938a), which may be used to obtain sufficiently large samples for dry-weight, ash, and microkjeldahl determinations. In 1952 Rawson indicated that the efficiencies of such nets deteriorated in one season's use from about 55 per cent to 25 per cent in spite of rigid precautionary measures.

Ricker (1938a) discussed the suitability of coarse-meshed nets in plankton collection and came to the general conclusion that for sampling adult entomostraca the No. 10-mesh silk was most satisfactory. The efficiency coefficient remained close to one over a series of 20 hauls taken in quick succession, and no significant change occurred over a period of service of a year and a half. Silk of No. 10 mesh is of course not suitable for the collection of phytoplankton or the smaller zooplankton forms. For example, Ricker states that three-quarters of the copepod nauplii pass through such mesh.

Tow nets of No. 20 silk show such wide fluctuations in efficiency that they cannot be recommended for the quantitative sampling of plankton. Coarser meshed nets may be used for collecting quantitatively the larger zooplankton forms, when the limitation as to the minimum size of organisms effectively sampled is recognized.

PLANKTON TRAPS

Since it was recognized that the tow-net method of collection was subject to such wide fluctuations in efficiency, Drs. Birge and Juday developed a plankton trap for quantitative sampling (Juday, 1916). This apparatus encloses a definite volume (ten litres) of water at any predetermined depth, and strains the organisms from the water by means of an attached silk net. Clarke (1942) describes a modification of the Juday trap in which the mechanical efficiency is considerably improved.

Theoretically the trap should take an accurate sample of the organisms at a particular point in the lake. That this may not be the case is indicated by Ricker (1938b), who suggests on indirect evidence that certain plankters, particularly *Daphnia*, may avoid the trap in daylight sampling. He suggests dusk or night sampling to eliminate this possible error.

Probably the most serious disadvantage of the Juday trap is that it is difficult or almost impossible to obtain a reliable measure of the average plankton population of even the deep-water area of a lake with this instrument because of variations in plankton abundance in both the vertical and horizontal planes. It may be possible, without undue labour, to overcome to some extent the effect of vertical differences in numbers of organisms by securing closely spaced trap samples from surface to bottom. On the other hand a number of replicate vertical series across the lake is also necessary in order to average the effect of horizontal variations in abundance. This adds so greatly to the labour of sampling and analysis that it becomes almost impossible to adequately sample even moderately

sized bodies of water. The great variation between samples experienced in the use of the Juday trap has caused Dr. John Neess (personal communication) to seriously discredit the use of the trap as a plankton sampler. He indicates "In Lake Mendota, we usually obtain coefficients of variation of the order of 200 per cent with the [Juday] trap. In the smaller ponds they go up to 4000 per cent".

If the Juday trap is used as the means of plankton collection, some effort must be made to overcome lack of randomness in spatial distribution, by taking replicate vertical series of samples at a number of stations in the open water of a lake at each sampling period.

CLARKE-BUMPUS SAMPLER

This plankton sampler described by Clarke and Bumpus (1950) is essentially a closing tow net equipped with a flow meter. Not only can the net be opened and later closed at any particular depth, but also the amount of water passing through the net can be determined by proper calibration of the flow meter. For the calibration to hold during the actual sampling by towing, the flow through the sampler must lie between the limits of one-half knot and four knots. Clarke and Bumpus indicate that within this range, which corresponds to a flow of from 3.25 to 26 litres per second, the rating for the meter is approximately four litres per revolution. Dr. John Neess, who has calibrated four such samplers by cementing them in a flume, has indicated in personal correspondence that such a rating held with a flow through the sampler from about 3 to 5.4 litres per second. At a flow of one litre per second the rotor stopped or operated intermittently and, between one and three litres per second, the flow rating varied inversely with the flow. This flow rating must be checked periodically for adequate quantitative results.

A No. 20 silk net apparently decreases the flow of water through the sampler below the range at which the flow rating is linear. This has been our experience in attempting to calibrate the sampler, with No. 20 silk, in the Algonquin Park lakes and is confirmed by Neess who indicates that he has given up entirely the use of the sampler with silk finer than No. 10. It would appear that the Clarke-Bumpus sampler is efficient when coarse-meshed nets are used, and is a definite improvement on coarse-meshed tow nets in that the amount of water strained is known. Equipped with No. 10 silk it is, of course, not suitable for phytoplankton collection nor the collection of smaller zooplankters (cf. p. 244).

It should be stressed that clogging of the pores of any silk net will reduce its straining capacity. Care must therefore be exercised with this instrument to maintain a flow above the minimum velocity required to operate the flow meter properly. This requires that the efficiency of the nets used must be checked after each haul to be sure that this minimum flow has been maintained. Clarke and Bumpus suggest that this is most easily done by determining the minimum number of revolutions which should be recorded for each minute of tow, and making sure that this minimum has been exceeded in individual hauls.

Although this procedure assures the accurate metering of water through the sampler it does not take into account the reduction in straining efficiency due to progressive clogging during a particular tow. Since this sampler must, like the

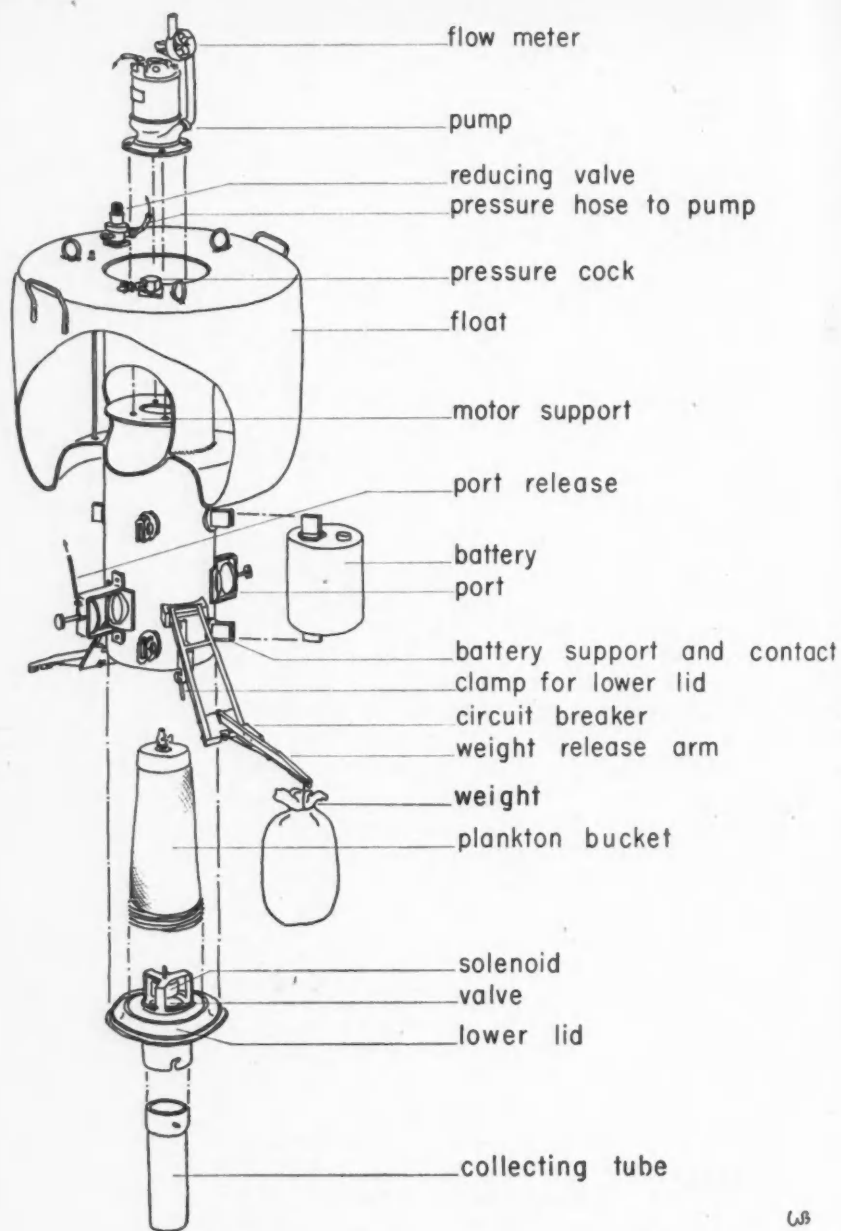


FIGURE 2. Exploded view of the 1952 model of Toronto plankton sampler.

tow net, decrease in straining efficiency with the length of haul and with the density of the population, the sample taken will not be truly representative of any population whose density differs at different positions along the haul. For example, in vertical or oblique hauls which begin at bottom, more water will be strained, per unit of distance towed, from lower levels than from upper strata, while the metre will record only the water strained for the complete haul. Thus a higher proportion of the population at lower levels will be sampled than that in upper strata. The effect of the changing efficiency of the net will not be as serious for coarse-meshed nets as for those with finer pores, but it should be considered either when making long tows or when sampling dense populations.

TORONTO SAMPLER

In order to overcome some of the criticisms of plankton samplers currently used, a new instrument was designed and built at the Ontario Fisheries Research Laboratory, Toronto. The first experimental model was tested in 1950 and considerably modified before further testing in 1951. A second instrument is being tested in 1952.

Support for this programme was given by the Research Council of Ontario. The sampler was developed in collaboration with Dr. F. E. J. Fry and Mr. Julian Kenny. We are indebted to Mr. James Ward for invaluable help in its construction.

CONSTRUCTION. Three main objectives were anticipated in planning the sampler. The first requisite was that the instrument sample the net plankton in the various strata of a vertical core of water from surface to bottom without change in efficiency during the haul. In order to maintain constant straining efficiency, water was to be pumped through No. 20 bolting cloth with sufficient head that any clogging effect was nullified. Secondly the amount of water strained in each haul was to be measured accurately. Thirdly the sample was to be obtained quickly and easily so that a number of hauls could be made at different positions on a lake in order to overcome horizontal differences in distribution.

A diagram of the 1952 model is shown in Figure 2. This instrument is essentially similar to the 1951 model except for the incorporation of certain improvements described on page 251.

The 1951 sampler was composed of two units, a floatation unit and the pump and power unit. The float was capable of supporting the weight of the apparatus plus a few pounds. It withstood 100 pounds of internal pressure and could be operated to depths of 200 feet when pressurized.

The pump was a 24-volt centrifugal aircraft fuel-booster pump, in which the armature, commutator and brushes were in a sealed housing. By pressurizing the housing with dry nitrogen, water could be prevented from entering. The pump was attached at the upper end of a five-inch cylinder 12 inches in length. A tapered plankton bucket was fitted within the cylinder, and to the lower opening was attached a spring-loaded valve which could be held open by a solenoid. Below the valve the collecting tube of clear plastic served as the

entrance for the water which passed through the bucket and out through the pump discharge tube. The collecting tube was eight inches long and had an aperture one and three-quarters inches in diameter. As it projected below the trap proper, it is believed that it exerted a minimum of disturbance to the organisms in the column of water sampled.

The power source for the pump and for the solenoid of the valve was composed of eight two-volt wet cells in cylindrical plastic cases. These were held on a circular shelf around the central cylinder and fitted within a housing



FIGURE 3. Toronto trap suspended from davit prior to submersion and descent.

attached to the float. The discharge tube from the pump was fitted with a flow meter with a direct drive to the counter. By calibration, a count of 7.56 revolutions was equivalent to a flow of one litre through the sampler. Two hinged legs attached to the battery shelf carried weights and a circuit-breaking mechanism.

In operation, the pumping and straining unit was enclosed within the annular float and clamped in position. The complete assembly was swung overboard on a winch and davit as shown in Figure 3. A five-pound weight was suspended on each release arm. This depressed the arm, closed the electric circuits and caused the pump motor to start and the solenoid to hold the valve in the open position. A reading was taken on the flow meter, the trap lowered into the water and the suspension cable released. The trap then sank freely.

As the sampler sank the pump drew water into the entrance tube and through the plankton bucket which was covered with No. 20 silk. The water passed

through the silk into the central cylinder around the bucket, thence through the pump and flow meter and out through the discharge tube above the sampler.

When the sampler touched bottom, the weights were released. This broke the electric circuits, the pump stopped and the intake valve snapped shut. The sampler, released from its weights, rose to the surface, was retrieved and hoisted aboard with the winch as indicated in Figure 4. A second reading of the flow



FIGURE 4. Toronto trap being raised from water after completion of haul. Water drainage is from cylinder surrounding plankton bucket.

meter was taken to determine the actual amount of water strained, and the bucket was removed and the contained organisms washed into the sample bottles.

The rate of descent, which had to be related to the capacity of the pump, was controlled by the weights attached to the release mechanism. These weights were predetermined by test runs so that the volume enclosed by the collecting tube as it descended was equal to the volume pumped per unit of time. This practically eliminated currents at the mouth of the entrance tube.

PERFORMANCE. No appreciable change in the rate of straining occurred during 15 minutes of continuous sampling with the sampler floating at surface. The total amount of water strained was in excess of 500 litres, which was 20 times the volume strained in a 20-metre haul. In addition, the surface water was rich in phytoplankton. Tests with half-charged batteries also showed no significant change in the rate of pumping. It is therefore believed that the straining efficiency remained constant in regular sampling.

Certain comparative series of samples taken at one station with the Juday trap and the Toronto sampler in 1951 indicated that the two methods were about equally efficient in capturing *Diaptomus*, *Cyclops* and *Tabellaria*. On the other

hand about twice the number of *Daphnia* were taken by the Toronto sampler as were taken by the trap.

Two comparisons have been selected as representative of those made in 1951. Four series with the trap when compared with four hauls with the Toronto sampler, all taken at one station, showed no significant difference in average number per ten litres for adults and nauplii of copepods or for three genera of rotifers (Table III). *Daphnia* was once more taken in significantly greater numbers by the Toronto sampler. That this might have been the result of avoidance of the Juday trap but not the sampler is a possibility. Such avoidance by *Daphnia* in daylight hauls with nets has been suggested by both Southern and Gardiner (1926) and Ricker (1938a).

TABLE III. Comparison of counts per 10 litres from four Juday trap series and four Toronto sampler collections taken at one station.

<i>Diaptomus</i>		<i>Cyclops</i>		Nauplii		<i>Daphnia</i>		<i>Polyarthra</i>		<i>Notholca</i>		<i>Anuraca</i>		
J	T	J	T	J	T	J	T	J	T	J	T	J	T	
63.7	59.0	51.7	47.5	222	181	35.8	44.6	118	97	50	52	154	254	
58.5	71.5	52.8	56.6	209	226	23.2	38.4	105	175	48	68	151	240	
67.1	56.6	64.4	38.4	214	210	32.1	41.3	144	114	61	61	222	240	
73.3	69.6	49.0	50.0	257	220	30.6	47.0	170	142	44	63	232	266	
\bar{x}	65.6	64.2	54.5	48.1	225	209	30.4	42.8	134	132	40.7	61	140	250
s^2	47.2		50.5		434		20.8		998		192		1013	
t	0.288		7.27		1.08		3.85		0.89		2.06		0.267	
Prob.	0.8		0.25		0.3		0.01		0.95		0.09		0.8	

The avoidance of the trap by *Daphnia* did not appear in a comparison of nine scattered series of Juday samples and ten Toronto samples horizontally spaced. All zooplankters except *Diaptomus* and *Cyclops* appeared to be taken equally well by both methods in these hauls (Table IV). The adult copepods were taken in significantly greater numbers by the Toronto sampler. It is believed that this might have been the result of the fact that the copepods were concentrated near surface and that the Toronto sampler took a disproportionate volume of water from the first metre in filling the inner cylinder. This defect of the sampler has been remedied in the 1952 sampler but in 1951 about four litres of water were taken from the upper metre in contrast to a volume of about one litre per metre at other depths. *Diaptomus* was 2.7 times as concentrated in the first metre as on the average for all depths. *Cyclops* was twice as concentrated in this upper layer and *Daphnia* was only 1.5 times as concentrated here as on

TABLE IV. Comparison of counts per ten litres from nine Juday trap series and ten Toronto sampler collections taken at different points on a lake.

<i>Diaptomus</i>		<i>Cyclops</i>		Nauplii		<i>Daphnia</i>		<i>Polyarthra</i>		<i>Notholca</i>		<i>Anuraca</i>	
J	T	J	T	J	T	J	T	J	T	J	T	J	T
129	275	100	159	322	382	86	102	69	73	92	84	49	60
\bar{x}	10324		2681		15557		1218		1075		553		393
s^2	3.13		2.49		1.05		1.05		0.026		0.65		1.21
t													
Prob.	0.008		0.03		0.3		0.3		0.35		0.5		0.2

the average. There was no comparable concentration at the time the comparisons were made at a single station. It appears, then, that the sampler collects as efficiently as the Juday trap.

ASSESSMENT. The Toronto sampler encloses a column of water, and pumps this volume of water through the straining silk as it sinks. The volume enclosed in sinking and that pumped are kept the same by calibration of the pump and by selecting the weights necessary to produce the correct rate of sinking.

The efficiency of straining and pumping has been found to be quite constant. The sampler with a 1.75-inch collection tube samples approximately one litre of water per metre depth. It requires about a minute and a half to complete a 20-metre haul. The sample can be removed and preserved on the way to the next sampling station, thus a number of total vertical hauls can be made rapidly at different stations over a lake.

The boat does not necessarily have to be anchored, except in heavy wind, although soundings should be made at each station so that checks can be made with the actual volume strained in each haul.

The instrument will operate in any depth over two metres or so, but is not recommended for depths less than from eight to ten metres.

The 1951 model was bulky and heavy. The total weight was about 80 pounds so a winch was required for handling, yet it was operated quite satisfactorily from a 13-foot plastic boat. The apparatus has been modified by rewinding the motor for 12 volts and using only four wet cells instead of eight. This change, together with the removal of the battery shelf and housing, reduces the weight considerably and allows for a much smaller floatation unit.

Since the 1951 sampler filled by straining water from the first-metre stratum, the later model has had ports added on the inner cylinder which obviate this error, by allowing the space between plankton bucket and cylinder to fill as it is submerged. These ports are closed automatically as the sampler begins its free descent. A column of water comparable in diameter to the total column is thus strained from the first-metre stratum.

It is believed that in the modified form the Toronto sampler will prove a satisfactory instrument for the quantitative collection of net plankton. Lack of randomness in both the vertical and horizontal planes can be minimized by taking several replicate hauls over the lake. The use of this instrument to ascertain the total net plankton of a series of lakes is planned for future investigations in Algonquin Park lakes.

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Factors Influencing the Growth of Phytoplankton

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ABSTRACT

In reviewing the literature dealing with the influence of physical, chemical and biochemical factors on the development and decline of phytoplankton pulses, this paper first outlines the seasonal cycle of phytoplankton pulses which occurs in many parts of North America and then presents evidence that this cycle is caused chiefly by the seasonal change in water temperatures resulting from the change in solar radiation. The way in which this cycle is sometimes obscured by irregular changes in phytoplankton numbers caused by local conditions of weather or nutrient supply is next illustrated. The climate, weather and chemical conditions are then resolved into the components light intensity, duration of illumination, temperature, concentration of nutrients, ionic balance and pH, and these are classified according to specific effects on the growth and survival of plankton algae as limiting, controlling

and lethal factors. The roles of factors originating within the organisms (i.e., autotoxins and antibiotics) are also considered. The discussion of laboratory investigations leads to certain criticisms of present culture methods and to some reinterpretation of observations obtained in some of these investigations. Finally, this paper illustrates the phenomenon of acclimation as it applies to phytoplankton, and it stresses the need for adequate prehistories of culture stocks in order that the results of different investigations can be validly compared.

INTRODUCTION

THE GROWTH of phytoplankton is influenced by factors of supply (limiting factors) and factors of control. Among the limiting factors are the intensity of light and duration of illumination which govern the supply of energy for photosynthesis, and the concentration of nutrient elements which constitute the structural units of carbohydrates. Temperature, ionic balance, concentration of catalysts, and probably pH may be the controlling factors which determine the rate at which phytoplankton can exploit the limiting factors.

At certain concentrations, the limiting and controlling effects may give place to lethal effects which influence the survival of the algae. For example, the concentration of iron, which may at times be a limiting factor in chlorophyll synthesis, may at other times be so low that the phytoplankton cannot manufacture sufficient chlorophyll to survive. At other times, the iron concentration may be so high as to be toxic. The extent to which an algal population grows depends, therefore, upon whether the species in question can survive the conditions in its environment, and upon the degree to which these conditions favour growth.

In the natural environment, the intensity of sunlight, the duration of illumination, and the water temperature vary with climatic and with weather conditions. By climatic conditions we mean here conditions which arise as a consequence of geographic position and the annual rotation of the earth (i.e., the seasonal cycle of changes in solar radiation and in water temperatures). Weather conditions are more irregular, of shorter duration and of more restricted geographic distribution. The climatic conditions may give rise to a comparatively regular cyclic succession of pulses of different algal species. Weather conditions, on the other hand, sometimes alter and obscure the underlying pattern determined by the climate.

The present paper is divided into two parts. The first part deals with the influence of climatic, weather and nutrient conditions on the growth and survival of plankton algae in certain temperate lakes in North America. More particularly, it is concerned with the way in which seasonal changes in water temperatures bring about a seasonal succession of different algal species, and with the way in which weather and nutrient conditions sometimes obscure the cyclic nature of the seasonal distribution of phytoplankton. The second part deals with particular aspects of the climate, weather and nutrient conditions (e.g., light intensity, duration of illumination, temperature, nutrient concentrations, pH, etc.) in terms of the concept of environmental factors (Blackman, 1905; Fry, 1947). In this second part, the data from field investigations are compared with data from laboratory studies of algae in cultures.

COMPARISON OF PHYTOPLANKTON POPULATIONS

The phytoplankton populations of many lakes in the North Temperate Zone are remarkably similar with respect to the proportions in which different algal groups (i.e., diatoms, green algae, blue-green algae, etc.) are represented in the annual crops.¹ Moreover, the changes which take place in the numbers of algae and in the relative proportions of the different species present, at different times of the year, are similar in these waters. In each case the seasonal distribution of phytoplankton is bimodal, with minimal numbers in winter and midsummer and maxima in the spring and autumn. When the spring and autumn maxima develop, the population is comprised chiefly of diatoms (Bacillariophyceae), and these make up the greater part of the total annual net phytoplankton crop. During the spring and autumn the Chrysophyceae (e.g., *Synura*, *Chrysosphaerella* and *Dinobryon*) may also be present in considerable numbers. Next in importance numerically to the diatoms in the total annual crop are the blue-green algae (Myxophyceae). These appear during the summer and reach their peak abundance towards midsummer, when the diatoms are at a minimum. Green algae (Chlorophyceae) are present during the summer also, but generally form a small part of the total annual crop. Fluctuations in numbers of green algae appear to be less regular than those of diatoms, blue-green and Chrysophyceae. Some observations from the literature will illustrate the comparison between phytoplankton populations just outlined.

The findings of Spencer (1950) for the Quabbin Reservoir, of Chandler and Weeks (1945) for western Lake Erie, and of Damann (1943) for Lake Michigan are listed for comparison in Table I. In each case diatoms comprised by far the greatest part of the total annual phytoplankton crop. Blue-green algae were next in abundance, while green algae and the Chrysophyceae formed only a small fraction of the total annual crop.

TABLE I. Comparison of the total annual crop of phytoplankton in Lake Erie and Lake Michigan.

	Percentage of total annual crop of phytoplankton		
	Quabbin Reservoir	W. Lake Erie ^a	Lake Michigan
Bacillariophyceae	79.6	79	90
Myxophyceae	15.4	14	3
Chlorophyceae	5.3	7	1
Chrysophyceae	1.2

^a Data for the 1942 phytoplankton crop.

Daily (1938) listed the algal groups appearing in Lake Michigan in order of abundance: Bacillariophyceae, Myxophyceae, Chlorophyceae, and Dinophyceae. Chandler (1942) found that diatoms constituted 98 per cent of the

¹Space dictates that only one type of seasonal distribution of phytoplankton can be discussed in detail in this paper. The type chosen is one which has been reported for lakes in many parts of temperate North America, and indeed in many parts of the North Temperate Zone as a whole. The few lakes cited in the following paragraphs were selected to show the widespread occurrence of this type of distribution in the United States and Canada, but many more examples could no doubt be added if space permitted. Considerable study has

total annual crop in western Lake Erie for 1940, and in 1941 they made up 50 per cent of the total annual crop and 70 to 100 per cent of the crop during the spring and autumn. In 1942 the spring pulse comprised 94 per cent diatoms, 4 per cent green algae and 2 per cent blue-greens, and the autumn pulse consisted of 76 per cent diatoms, 4 per cent greens and 20 per cent blue-greens (Chandler and Weeks, 1945). Proportional relations similar to those given by Spencer, Damann, Daily and Chandler were obtained for certain lakes in Algonquin Park, Ontario (McCombie, 1950) and for Cultus Lake, British Columbia (Ricker, 1937). In the Algonquin Park lakes the general picture (for 1946-1949) was similar to that outlined at the beginning of this section.

The Quabbin Reservoir, Lake Erie, Lake Michigan, the Algonquin Park lakes and Cultus Lake are all in the North Temperate Zone, where there is a characteristic seasonal change in water temperature which follows the cycle: cold—moderate—warm—moderate—cold. This change in water temperature is probably a prime factor influencing the seasonal distribution of phytoplankton, and the different species of algae apparently develop pulses as the water temperature becomes favourable to them. This theory is further supported by the fact that differences in the time of development of algal pulses from year to year frequently correspond to the attainment of favourable temperatures at different times. In 1948, for example, the seasonal distribution of diatoms and chrysomonads in Costello Lake, Algonquin Park, was unimodal, and the development of the spring pulse of these forms was apparently extended so that the spring and fall maxima coincided. This was probably due to the fact that Costello Lake warmed up very gradually that year and did not attain temperatures which might cause diatom populations to fall to a midsummer minimum (McCombie, 1950).

Sometimes, however, the bimodal character of the seasonal distribution of phytoplankton is more or less obscured by non-cyclic events which apparently result from local conditions, such as cloudy weather or scarcity of nutrients. Several days of cloudy weather may arrest the development of algal pulses, by reducing both the light intensity and the period of effective illumination. Algal blooms, on the other hand, appear to result from light, temperature and nutrient conditions favouring growth simultaneously (McCombie, 1950). Thus, in the Algonquin Park lakes, green and blue-green algae normally appear only in small numbers and only when the water is warm. However, in 1947 and 1948 these algae appeared in bloom concentrations in those Algonquin Park lakes which were fertilized while the water was warm. That these blooms depended on sunlight, as well as upon the abundance of nutrients and the warm temperatures, was shown by the fact that they declined markedly during periods of cloudy weather.

been devoted to this type of phytoplankton distribution owing, in a large measure, to the fact that many of the lakes concerned have presented problems in fish production or in sanitation and water supplies. This type of distribution is therefore of practical as well as theoretical interest to the aquatic biologist. The reader will realize, however, that the phytoplankton in other types of waters (e.g., smaller ponds, bogs, streams) or in other geographic regions (e.g., the tropics or the arctic) will probably present characteristics of composition and distribution which are not discussed here.

PHYSICAL AND CHEMICAL FACTORS

Blackman (1905) gave the first valid demonstration of the relation between an activity and a *single* one of the various factors affecting it. In considering the factors influencing the rate of photosynthesis by leaves, he pointed out that these fall into two categories, namely (1) those which limit the supply of material or energy, and (2) those which affect only the rate of the process. Accordingly, the carbon dioxide supply and light intensity belong to the first category, while temperature belongs to the second. Blackman considered both types of factors as *limiting*, and described their action thus: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor". That is, if the carbon dioxide supply, temperature and other factors were sufficient to support an assimilation rate of 10 cc. of CO_2 /hour, but the light intensity sufficed for only 5 cc./hour, then the light intensity would limit assimilation to 5 cc./hour. If the light intensity were raised to a level which would permit an assimilation of more than 10 cc./hour, light would cease to be a limiting factor and one of the other factors would assume that role. Blackman objected to the usual concept of an "optimum" concentration lying between the minimum and lethal levels and giving the greatest rate of metabolism or activity. He demonstrated that the "optimum" reported by many investigators was probably the result of their failing to recognize that metabolic rates fall off more and more with time, as concentrations of a factor approach lethal levels. As these investigators usually allowed considerable periods to elapse between the introduction of the organisms to experimental conditions and the first measurement (to allow handling stimuli to disappear), they did not attain initial rates but slower rates instead. Blackman indicated, however, that a real optimum might be obtained in certain instances. Such an optimum might occur, for example, if the factor affected both constructive and destructive processes in metabolism, and the balance changed in favour of the destructive as the concentration passed the optimum level. Finally, he attributed the decline of metabolic rate with time at higher concentrations of factors to a "time factor". For factors at lethal concentrations this "time factor" would, of course, be only one aspect of the dosage-exposure relation.

Since Blackman presented his classic paper, the classification of environmental factors according to the specific effects on metabolism and activity has been extended and further refined. Fry (1947) recognized six categories of environmental factors, namely: *limiting*, *controlling*, *lethal*, *accessory*, *directive* and *masking factors*. In this paper he defined as *limiting factors* those which Blackman had shown to act through supply of material or energy, and as *controlling factors* those which merely affected the rates of metabolism and activity. Factors which are present at lethal concentrations (or intensities) were termed *lethal factors*, and more precise methods of determining the lethal levels were discussed. Fry emphasizes that several controlling factors may act at the same time, but only one limiting factor can act at a time. Many if not all factors may become lethal at concentrations beyond certain upper and lower lethal limits. Within these lethal limits lies the *zone of tolerance*, and beyond these limits lies

the *zone of resistance* (Fry, 1947). If the concentration is within the zone of tolerance it will not kill the organism. If, however, the concentration is within the zone of resistance it will eventually kill the organism, and the time required for the organism to die is a function of the lethal dosage.

LIGHT

The amount of solar energy available to phytoplankton is a *limiting factor* when it sets a limit to the supply of energy for photosynthesis. Although this limiting effect is manifest directly in the photosynthetic rate, any factor which limits photosynthesis is likely to restrict other processes in the plants. Thus the growth rate will be indirectly affected by the supply of light energy. However, photosynthesis, protoplasm formation and cell division may be more or less independent over short periods of time, so that there can be a lag between the fall in photosynthetic rate and the slowing up of growth. The amount of light energy available for the photosynthesis of phytoplankton will be a function of the colour and intensity of the light and the duration of illumination. Light may also be a *lethal factor* when operating at high intensities or at ultra-violet wave lengths.

COLOUR OF LIGHT. At present the evidence indicates that plants can carry on photosynthesis in light ranging from 3,650 Å to 7,500 Å though the greatest efficiency is found in orange-red light (6,550 Å). Klugh (1925), using Wratten filters to obtain light of different colours, found that *Volvox aureus* and *Closterium acerosum* reproduced most in red light, less in blue light, and not at all in green light. Dangeard (1927), using microspectra as well as glass filters as sources of coloured light, found that growth and multiplication of green algae, blue-green algae and diatoms took place only in red light. Other radiations affected the algae as though they were in complete darkness, i.e., the algae did not multiply. The high efficiency of red light was put to good use by Ketchum and Redfield (1938), Scott (1943) and others when they used "neon" tubes in culturing phytoplankton. Such tubes give light of high photosynthetic efficiency with a minimum of heat.

Although red light is generally considered to be most important for plant metabolism, some workers have reported that other wave lengths appeared more important. Grintzesco (1902) claimed that the development of colonies of *Scenedesmus acutatus* was more active under blue-violet light than under red-yellow light. Nadson (1910) exposed cultures of *Stichococcus bacillaris* to sunlight filtered through solutions of calcium dichromate and of cupra-ammonium salts, and reported that red light was unfavourable, as it not only inhibited growth but even destroyed cellular organization. He observed that the cultures exposed to green light showed about the same growth as those in full sunlight. In contrast to Nadson, Meier (1936) found that the abundance of *Stichococcus bacillaris* increased threefold in blue light (compared with controls kept in the dark) and twofold in yellow and red light, whereas green light proved destructive.

The variety and contradiction in the results of investigations on the effects of different colours of light can be attributed to (1) differences in the responses of different species, (2) differences in the levels of other factors, (3) differences in techniques and apparatus. The earlier workers (e.g., Grintzesco and Nadson) used coloured chemical solutions, which have since been shown to be quite unsatisfactory. Dangeard (1927) demonstrated that such solutions might transmit "parasitic" rays (i.e., rays other than those responsible for the visible colour of the solution), which could influence the metabolism of the plants. Klugh (1925) and others have stressed the importance of adjusting the light transmitted by filters to the same intensity before comparing the effects of colours upon plant metabolism. The later workers have used more satisfactory filters giving narrower colour bands, and have had better means of analysing and measuring the transmitted light.

Ultra-violet radiations are probably *lethal factors* to phytoplankton, as they are to bacteria, entomostraca and other micro-organisms (Klugh, 1929, 1930a and b; Welch, 1952). The fact that ultra-violet light is destructive to organisms which do not perform photosynthesis suggests that its lethal effects on phytoplankton may be manifest through other metabolic processes. Although there appears to be no specific information about the intensity or exposure required for ultra-violet light to kill plankton algae, the experiments of Gerloff *et al.* (1950a) indicate their resistance to some extent. They found that, by exposing cultures of blue-green algae to ultra-violet light (2,750 Å) for 20 to 30 minutes, they could obtain subcultures which were free from bacteria but still contained viable algae. In spite of the fact that ultra-violet light is probably lethal and that it penetrates for only a short distance into water, Loosanoff (1951) equipped a greenhouse constructed for mass culture of algae with special glass which permitted penetration of "the ultra-violet rays needed for growth of phytoplankton". However, he gives no reason for assuming these rays are necessary.

INTENSITY OF LIGHT. Light intensity may be a *limiting factor* or a *lethal factor* according to its level. As a limiting factor, light determines the rate of supply of energy for photosynthesis. When the intensity is such that the energy supply is insufficient for the plant to exploit fully the other factors conditioning the process (e.g., temperature and carbon dioxide supply), then that intensity is limiting. In Blackman's terms, light intensity is in this case the "slowest" factor. Beyond certain upper and lower limits, light intensity becomes a lethal factor.

One important aspect of Blackman's concept which has had to be modified is the way in which he considered that one factor of supply (e.g., light intensity) might succeed another (e.g., carbon dioxide supply) as the limiting factor in photosynthesis. His diagrams suggest that, when the carbon dioxide supply ceased to be limiting and the light intensity assumed that role, the point would be marked by a sharp transition. However, investigators have since established that there is no such sharp changeover, but rather a region of transition where one factor becomes less limiting and another becomes more so.

The limiting effect of light intensity on the photosynthetic rate of *Chlorella* has been well demonstrated (Myers and Clark, 1944; Myers, 1946a and b).

Myers developed a special apparatus which permitted him to dilute his stock cultures with fresh medium when they attained a certain density, in order to prevent the nutrient supply being appreciably depleted. From these stock cultures he took samples for determination of photosynthetic rates in Warburg vessels. By measuring the photosynthetic rates of samples, each kept at a different light intensity, Myers showed that photosynthesis accelerated with increasing light intensity up to a certain level. Further increase in intensity beyond this level produced no further acceleration in photosynthesis, and the rate became constant. At those levels where the photosynthetic rate was related to the light intensity, the light intensity was the limiting factor. At the levels where the photosynthetic rate was constant, some factor other than light must have been limiting. While the limiting effect of light intensity is manifest directly in a restriction of the photosynthetic rate, it may be expressed indirectly in a limitation of the growth rate. The photosynthetic and growth rates in Myer's experiments were closely related. He found that the level below which light intensity became limiting to photosynthesis varied from about 200 to 400 foot-candles, depending upon the light intensity to which the stock cultures were acclimated. The great value in Myer's work lies in the fact that he used stocks which were always growing in a medium of relatively constant composition, and he made his observations over short time intervals so that the medium in the Warburg vessels did not change appreciably.

Other investigators have attempted to determine quantitatively the effects of light intensity on the growth and survival of phytoplankton by measuring the populations each day as they developed in culture vessels. On the basis of such observations, Rodhe (1948) reported that the growth rate of *Melosira helvetica* increased with rising light intensity up to a level which he termed the "optimum", and then declined with further increase in intensity. Rodhe suggests that this "optimum" level is movable and that its value depends on the duration of illumination and the temperature. There is, however, a serious objection to this type of experiment where the phytoplankton populations are observed as they build up over several days in culture vessels. The objection is that the nutrient supply may become reduced and cause the growth rate to fall off at an artificial optimum. Rodhe found that the growth declined with increase in the period of illumination and in temperature. The fact that both of these factors would increase the demand on the nutrient supply suggests that the nutrients in this case were quite probably exhausted before the light reached a true optimum.

The harmful effects of high light intensity are expressed primarily through a decline in the photosynthetic rate of plants (Rabinowitch, 1945). However, if a plant (e.g., a phytoplankter) is exposed to light intensities which inhibit photosynthesis for a sufficient time, other phases of its metabolism will be affected, and death will follow. Damann (1943) recorded that the growth rate of *Chlorella* increased with increasing light intensities of 25, 75, 300 and 500 f.c., but at 1,000 f.c. the number of cells produced was less than at 500 f.c. Hence the light intensity appeared to be an inhibitory or lethal factor at 1,000 f.c. Cook (1949), citing Myers and Burr, points out that "*Chlorella* exposed to high light intensities will

grow rapidly at first, then at a diminishing rate until injury takes place and death of the cell occurs". This suggests that the effect of high light intensity is lethal rather than simply inhibitory.

When the light intensity falls to a level so low that the photosynthesis of the plant can just satisfy its respiratory requirements, the light intensity will have reached its lower lethal limit. At intensities below this limit, the cell will die within a period of time which will be shorter the lower the intensity. This lower lethal level is usually termed the *compensation point* (Pettersson *et al.*, 1934; Clark, 1939; and others).

If a decrease in light intensity occurs while the population of a plankton alga is building up, the development of that population may be slowed up or arrested. Some indication of the extent to which such a decrease in light intensity may influence the development of populations of *Asterionella formosa* has been shown by Lund (1949). In one experiment, cultures of this diatom were grown in full light for four days and then shaded between the fifth and tenth day. As a result of the shading, growth ceased and the number of cells in the cultures remained constant from the fifth to tenth day. Upon removal of the filters, growth was resumed, and the rate quickly returned to the value it had during the first four days. Dull weather entails reduced light intensities, with the result that development of phytoplankton pulses may be curtailed. The effect of dull weather will, of course, depend not only upon the degree to which the light intensity is decreased, but also upon the period over which it is reduced. This time factor will be discussed further in the section on the duration of illumination.

In the natural environment, the position of the sun, the colour and turbidity of the water, and the distance between the organism and the surface will all determine the quality and intensity of light reaching the phytoplankton. As light differs qualitatively and quantitatively at different depths, the photosynthetic activity of the plankton algae varies with depth. Furthermore, if organisms in the trophogenic zone are circulating, their photosynthetic rate may be continually changing. The phytoplankton themselves may also alter the amount of light penetrating into their environment by increasing the turbidity and colour of the water as their numbers increase (Wiebe, 1930; Cook, 1949). Blooms of *Anabaena* concentrated near the surface of certain Algonquin Park lakes reduced the penetration of light to a marked degree (McCombie, 1950). When such a bloom was at its height in Kearney Lake, in midsummer of 1948, only 1.0 per cent of zenith sun penetrated to 1 metre, whereas in 1949 when the algal population was fairly heavy but not in bloom, 14 per cent of zenith sun penetrated 1 metre. This seems to have reduced the summer crops of diatoms and chrysomonads in Kearney below even their usual midsummer minimum, by depriving these species of light.

DURATION OF ILLUMINATION. While the rate at which carbon dioxide is assimilated by phytoplankton depends upon the light intensity (when intensity is a limiting factor), the total amount of carbon dioxide assimilated in a given period depends also upon the length of time the organisms have been effectively illuminated. Effective illumination may be defined as illumination at intensities above

the compensation point. A portion of the carbon dioxide assimilated will be reflected in increased abundance of phytoplankton. There are two chief ways in which the period of effective illumination varies in the natural environment: the number of hours sunshine per day increases with the approach of summer, and the number of hours of sunshine per day may be reduced by cloudy weather. It should be appreciated that the duration of effective illumination is not a limiting factor in Blackman's sense of the term, since it affects the *total amount* of photosynthesis rather than the *rate*.

The increase in length of day with the approach of summer favours increased production of phytoplankton. The difference between the winter, spring and summer productions of phytoplankton is undoubtedly in part a function of the average length of day in each season. However, the crash of spring pulses before June 21 (McCombie, 1950) indicates that other factors (e.g., scarcity of nutrients, lethal temperatures, etc.) tend to prevent the phytoplankton from benefitting fully from the longer days.

On the other hand, short-term reductions in the average number of hours of sunshine per day, such as attend cloudy weather, may result in the phytoplankton failing to exploit fully the nutrient and temperature conditions when these factors are favourable. The prevalence of sunny or cloudy weather for a period of several days appears to be one of the most important factors determining whether phytoplankton pulses will build up, or die off, respectively. Purdy (1937) observed that the number of cells of *Oocystis* cultured in daylight was greater when the number of hours of sunshine per day was greater between sampling dates, whereas during dull periods the production dropped. Data for the Algonquin Park lakes suggest that there is some correlation between phytoplankton pulses and periods of sunny weather (McCombie, 1950). According to Verduin (1951), cloudy weather may have caused the spring diatom pulse in western Lake Erie to develop one month later in 1950 than in 1949.

TEMPERATURE

Water temperature may be a *controlling factor* or a *lethal factor* for phytoplankton, depending on the temperature level in the environment. As a controlling factor, the water temperature controls the rates of metabolism and growth of phytoplankton, but unlike a limiting factor it does not act through restriction of the supply of energy or materials. Rather, water temperature sets the tempo at which the phytoplankton can exploit the limiting factors (e.g., light and nutrient conditions). The controlling effect of temperature is expressed between the upper and lower lethal limits of temperature. It is between these limits that phytoplankton are able to develop pulses, provided that other factors such as nutrient and light conditions are also favourable. When temperature is a controlling factor, the rates of metabolism and growth increase with rising temperature up to a certain level and then decline with further rise in temperature. The cause of this decline is, of course, unknown but some writers suggest that it is due to a shift in the balance of constructive and destructive metabolic processes at higher temperatures. The temperature at which the rate of metabolism or of growth is

greatest is the optimum temperature, in the sense Blackman was prepared to accept. Above the optimum lies the upper lethal limit, beyond which the phytoplankton will die from the effects of temperature. Below the lower lethal limits also, phytoplankton will die of adverse temperature effects.

Among the evidence for the controlling effect of water temperature on the growth rate of phytoplankton are the frequent reports that the increase in spring phytoplankton abundance is correlated with rising water temperature (Daily, 1938; Spencer, 1950; and others). It is difficult, though, to tell the degree of this correlation from field observations, because both the temperature and phytoplankton numbers rise during the spring and early summer. Consequently there is a multiple correlation between temperature, time and growth, and it is difficult, if not impossible, to separate out the effect of the time factor under natural conditions.

Lund (1949) has shown, to some extent, the degree of correlation between temperature and the abundance of *Asterionella formosa* in cultures. Here the time factor was eliminated by growing duplicate sets of cultures at different temperatures simultaneously. In the cultures which were cool to begin with, the growth was slow during the first four days, but accelerated quickly when the temperature was raised. In the cultures which were warm to begin with, the growth rate was high (after an initial acceleration from zero), but declined rapidly when the temperature was lowered. During the last two or three days of observation the growth rates in both sets of cultures coincided approximately and fell off, suggesting (as Lund points out) that the nutrient supply had become exhausted.

The temperature-growth relation could account (at least in part) for the observation that spring phytoplankton maxima are often greater than the autumn maxima (Welch, 1938; Ricker, 1937; Lund, 1949; and others). During the spring the development of phytoplankton pulses will be accompanied by a rise in water temperatures, which tends to accelerate the growth rate. In contrast, the development of the autumn pulses will be accompanied by a decrease in temperature, which will slow down the growth rate.

Within its particular temperature limits, a species of plankton algae will be able to develop a pulse, provided other factors are favourable. Beyond the lethal limits, adverse temperature effects will tend to reduce the numbers of the algae concerned, even though other factors favour increases. As pointed out earlier in this paper, the occurrence of pulses of different algal species at different times of year appears to be correlated with their temperature tolerances. Diatoms and chrysomonads, for example, flourish at cool to moderate spring and autumn temperatures, but decline markedly in number in cold winter waters or in warm summer waters. Green and blue-green algae flourish in warm waters, but are scarce or entirely absent when the water is cool or cold. The following three paragraphs list some of the evidence supporting these conclusions and a few of the tolerance data obtained from field and laboratory investigations.

Field investigations give rise to four lines of evidence of the importance of temperature tolerance and optima in the seasonal distribution of phytoplankton.

Some of these points have been mentioned in preceding sections, but they are repeated to summarize the evidence as a whole. First, the appearance of diatoms and chrysomonads in the spring and autumn, and of green and blue-green algae in the summer, occurs consistently each year in many lakes of the temperate zone. Secondly, the temperature at which the pulses of diatoms and chrysomonads begin to develop in the fall is approximately the same as the temperature at which the spring pulses decline. In the late summer or autumn the pulses of green and blue-green algae decline markedly as the water cools below the temperature at which these pulses began to form (McCombie, 1950). Thirdly, although some authors have reported that the decline of phytoplankton numbers during the summer was due to exhaustion of nutrient (in particular, phosphorus), there is evidence that this decline is not always correlated with a decline in nutrients (Welch, 1952). Welch points out that Birge and Juday, working on Wisconsin lakes, found that, in spite of the fact that there were fairly large crops of phytoplankton present during the summer, in most cases the phytoplankton did not appreciably alter the phosphorus supply. In the Algonquin Park lakes which were fertilized, the decline of diatoms and chrysomonads took place during the summer, even though the nutrient had been increased and light conditions were sufficient to support blooms of green and blue-green algae (McCombie, 1950). Similarly, the decline of the blue-green algae in these lakes took place in the autumn, although the nutrient and light conditions were satisfactory for development of autumn pulses of diatoms and chrysomonads. Fourthly, variations in the time at which particular pulses of algae develop from year to year or lake to lake are apparently correlated with different rates of warming or cooling in each case. That is, the water may reach a temperature suitable to a particular alga sooner in one year than in another, as a result of different weather conditions. Or the water in one lake may reach temperatures suitable to the alga sooner than in another lake, because of morphometric differences which permit more rapid warming.

Table II presents some approximations of the temperature tolerances of certain phytoplankton, as determined from field investigations on Algonquin Park lakes (McCombie, 1950). The average temperature of the epilimnion was taken as an approximation of the temperature of the trophogenic zone on these lakes. The data given summarize observations made on several lakes during 1946, 1947 and 1948, and further observations made in 1949 and 1950 are in general agreement with these.

TABLE II. Temperature ranges within which various algal pulses formed in the Algonquin Park lakes in 1946, '47 and '48.

Alga	Temperature range (in terms of the average epilimnial temperature)
Diatoms: <i>Tabellaria</i> and <i>Asterionella</i>	14 to 21°C.
Chrysomonads: <i>Dinobryon</i>	11 to 19°
<i>Synura</i> and <i>Chrysosphaerella</i>	15 to 18°
Blue-green algae: <i>Anabaena</i>	17° to ? ^a

^a Upper limit unknown, but probably above 25°C.

These data indicate that the diatoms concerned (which make up the bulk of the spring and fall maxima) develop pulses in cool to moderate waters, whereas the blue-green algae thrive within a higher range. Damann (1943) obtained similar results for Lake Michigan phytoplankton, though there were differences in his absolute values (Table III). These differences are probably due largely to differences in plankton collection methods, temperature measurements, etc.

Table III sums up some of the temperature-tolerance data which Damann (1943) obtained when he cultured phytoplankton from Lake Michigan. In addition some of his field observations are included for comparison. Damann's laboratory results agree in general with the results from his field studies. On

TABLE III. Temperature ranges within which various plankton organisms from Lake Michigan survived. Data taken from Damann (1943).

Temperature of culture °C.	Algal form dominating the culture	Corresponding condition observed in the lake, within same temperature range
10 to 13	Diatoms: <i>Tabellaria</i> , <i>Asterionella</i> , <i>Fragilaria</i> , and <i>Synedra</i>	Spring maximum comprised chiefly of diatoms
17 to 22	Green algae, and blue-green algae in small numbers	Summer minimum, chiefly green and blue-green algae present
30 to 32	Blue-green algae	Such temperatures not observed in the lake

culturing phytoplankton from certain Swedish lakes, Rodhe (1948) obtained results similar to those observed for the Algonquin Park lakes and for Lake Michigan. He found, for example, that it was not advantageous to culture the diatoms *Asterionella formosa* and *Fragilaria crotonensis* at temperatures above 15°C., and that the green algae *Scenedesmus*, *Ankistrodesmus*, *Chlorella*, *Pediastrum*, and *Coelastrum* grew best at 20-25°C. Gerloff (1950a) succeeded in culturing several species of blue-green algae, including the common plankton forms *Nostoc*, *Aphanizomenon* and *Gleocapsa*, at 25°C., although his experiments at the time were not aimed at determining the actual tolerance range.

The evidence just presented indicates, therefore, that there is considerable justification for dividing the phytoplankton of temperate-zone lakes into warm- and cool-water forms. However, it is intended that this division should apply only to some of the more predominant species found in temperate-zone lakes, and not to phytoplankton as a whole. There are in all probability planktonic diatoms which tolerate warm waters, and planktonic green and blue-green algae which will flourish in cool waters. The phytoplankton of tropical waters are probably forms which tolerate higher temperatures.

Obviously there is much need for further investigations of the temperature tolerances of plankton algae. In such investigations, laboratory cultures have a number of advantages over field surveys. Among the more important advantages is the fact that uniformity of temperature and of distribution of the organisms can be attained in cultures. As a result, the investigator is not obliged to fall back on such approximations as the average temperature of the epilimnion, or

the average number of organisms in the epilimnion. In field studies it is difficult to determine the temperatures at which the algae are flourishing, when both temperature and distribution of organisms vary with depth. On the other hand, caution should be taken when culturing phytoplankton to see that nutrient or light conditions do not become limiting. This danger is especially imminent when one is seeking to find the upper limits of temperature tolerance, because higher temperatures tend to accelerate metabolism and consequently place a higher demand on the factors of supply.

Before turning from a consideration of the roles of light and temperature to those of nutrients, one more point should be considered. Although the argument presented up to this point has been that the seasonal distribution of various species of plankton algae depends largely upon their temperature tolerances, there is also a possibility that it depends to some extent upon their light-intensity tolerances. Each species of alga may possibly have a characteristic zone of tolerance for light intensities, just as it has a characteristic temperature tolerance. Rodhe (1948) and Vollenweider (1950) point out that Findenegg, on the basis of many years' study of a dozen Austrian lakes, classified the phytoplankton with regard to both light-intensity and water-temperature relations thus:

	Schwachlichtformen	Starklichtformen
Kälteformen	Winterplankter	Frühjahrsplankter
Wärmeformen	Herbstplankter	Sommerplankter

Recently, Wohlschlag and Hasler (1951) suggested that the development of diatom pulses during the spring and autumn, and of green and blue-green algae pulses in summer, in a temperate-zone lake may be due to these being weak-light and strong-light forms respectively. They took mud samples from Lake Mendota during the winter and cultured them in daylight at 20°C. Half of the culture flasks were covered with brown paper. The phytoplankton content of all flasks was negligible to begin with. After 18 days there was growth in all flasks, though the phytoplankton was much more abundant in the unwrapped flasks. The unwrapped flasks contained mostly green and blue-green algae and smaller numbers of diatoms, whereas diatoms comprised most of the crop in the wrapped flasks. Three objections might be raised to this experiment. First, 20°C. is not a temperature favourable to diatoms, according to the experimental determinations of Damann and Rodhe (see above). Secondly, Wohlschlag and Hasler do not indicate whether the covering interferes with the warming of the cultures by sunlight. It may be that the unwrapped cultures warmed up more so that their temperature was more favourable to green and blue-green algae. Thirdly, their use of colorimetric determinations of the chlorophyll content of mixed cultures of diatoms, green algae and blue-green algae may not have given a sound basis for comparison of crops, because of the differences in pigmentation involved (Tucker, 1949). Nevertheless, the possibility that the seasonal distribution of different species of phytoplankton depends (at least in some instances) upon their light-intensity tolerances cannot be ruled out, because of the present scarcity of laboratory demonstrations. Moreover, it is difficult (if not impossible) to state

with certainty from field observations that the decline of any algal pulse is due to unfavourable temperatures rather than to unfavourable light intensities, because the seasonal changes in water temperatures and light intensities are related. The argument will only be settled when more numerous and precise data are available for the tolerance limits of water temperatures and light intensities, and for the temperatures and light intensities encountered in the lakes.

NUTRIENTS

The concentration of any nutrient substance may be a *limiting factor* or a *lethal factor* according to its level in the environment. When the concentration of any nutrient is a limiting factor, it sets a limit to the material available for metabolic processes. This in turn influences the growth and survival of the phytoplankton. The concentration of a nutrient will become limiting to a metabolic process when that nutrient becomes scarce relative to the supplies of other nutrients and of energy required for the process. This is better explained by postulating a simple case in concrete terms. Suppose therefore that, for every molecule of carbon dioxide assimilated by the phytoplankton, one molecule of water, one atom of some catalyst (say iron) and four quanta of light energy are required. If there were 10 molecules of carbon dioxide, 10 molecules of water, 40 quanta of energy, but only five atoms of iron present, the assimilation rate would be proportional to five. That is, the assimilation rate would be limited by the metabolic requirement on shortest supply, namely the iron. If the iron supply were to increase continuously until there were 10 atoms available, a level would eventually be reached above which this supply would become less and less limiting. At the same time the carbon dioxide and light intensity would begin to be more limiting. That is, there would be a region of gradual transition where the carbon dioxide and light intensity succeeded the iron supply as the limiting factor. The supply of a nutrient substance becomes a lethal factor when the concentration is so low that the phytoplankton starves, or when it is so high as to be toxic. Between the limits of starvation and of toxicity lies the zone of tolerance, within which the algae will not succumb to adverse effects of the nutrient concentrations concerned. Within the zone of tolerance the nutrient supply may be sufficient, or more than sufficient for the organisms' immediate requirements. Beyond the lethal limits the plankton will die of adverse effects of the nutrient concentration, and the time to death depends on the degree of starvation or of toxicity. In the case of starvation, the resistance will depend, among other things, upon the amount of reserve materials accumulated within the plants.

In addition to acting as a limiting factor and a lethal factor, the concentration of chemical elements or ions may in some cases be a *controlling factor*. This controlling effect arises from the fact that the presence of some elements may control the rate at which the plants can take up and metabolize other elements. One way in which the concentration of an element may control the rate of utilization of another is by altering the permeability of the cell. The ions of some elements, for example, may precipitate the proteins in the cell membrane,

and thus reduce its permeability. Other ions may have the opposite effect, causing a greater dispersal of the constituents in the cell membrane and increasing the permeability. A third way in which the ions of one element might alter the permeability of the cell to the ions of another element is by competing with it for a position on the cell membrane, or perhaps for a position on some protoplasmic constituent. Elements which show *antagonism* or *synergism* may show such controlling effects (i.e., reduction of permeability). Among these elements are calcium, magnesium and potassium, which are among the most abundant elements in natural waters (Vollenweider, 1950). Another way in which the concentration of one element can control the rate of uptake of another element is by acting as a catalyst for a metabolic process. It is possible that some of the "trace" or "minor" elements may control the rate of uptake of the "major" elements in this way. It should be recognized that the concentration of an element or ion can be considered to be a controlling factor only in so far as it affects the rate of a metabolic process without acting as a factor of supply for that process. If the element or ion becomes incorporated into some product of the metabolism then the concentration, if it affects the metabolic rate, must be regarded as a limiting factor. However, an element or ion may sometimes control certain processes and limit others simultaneously. Thus when the magnesium concentration regulates the permeability of the cell membrane, it may control many if not all of the processes going on within the cell. On the other hand, the magnesium supply may at times limit the process of chlorophyll formation, since this element actually forms a part of the chlorophyll molecule.

Each species of alga probably has characteristic upper and lower tolerance limits for concentrations of different nutrients. Chu (1943) found, for example, that 0.4 ppm. of nitrate-nitrogen supported the growth of *Botryococcus* sp., but did not support growth of *Pediastrum* sp. which required a minimum of 0.7 ppm. Presumably the toxicity levels are also different, but the evidence for this is less satisfactory at present. Within the zone of tolerance, the nutrient concentrations at different values may (1) merely satisfy the requirements to avoid starvation, (2) prevent starvation and permit growth at a limited rate, and (3) permit growth at a rate unlimited by the nutrient supply in question.

The reduction of the nutrient supply casts some doubt on many data on growth-nutrient relations obtained from culture experiments. This applies particularly to data on growth rates or population data obtained by observing cultures over relatively long periods (several days in some cases). Obviously, if the supply of any nutrient is reduced by the metabolism of the algae, the concentration of that nutrient at the beginning of the experiment does not give a true picture of the conditions at the end of the experiment. Thus, for example, one might attempt to measure the growth rate of an alga at some limiting level of a particular nutrient by innoculating a medium containing that limiting concentration, and then recording the growth each day. As time progressed, however, the concentration of the nutrient would drop, becoming more and more limiting, and correspondingly the growth rate would drop. Consequently at the end of

the experiment both the nutrient conditions and growth rate would differ from those at the beginning.

CARBON. Carbon is essential to the construction of all carbohydrate and protein molecules. Since the photosynthetic mechanism is responsible for the assimilation of carbon by plants, a limiting carbon supply will affect the rate of photosynthesis directly. Rates of other metabolic processes and of growth will be affected indirectly.

Carbon is present in the aquatic environment as free carbon dioxide (CO_2), half-bound carbon dioxide (HCO_3) or bound carbon dioxide (CO_3). Phytoplankton can assimilate free and half-bound carbon dioxide directly, and bound carbon dioxide indirectly when carbonates dissociate. Carbonates serve to store up carbon dioxide when it is plentiful and release it when it is scarce (Welch, 1952). Some writers suggest, therefore, that phytoplankton crops of hard-water lakes tend to be heavier than those of soft waters, since the carbon dioxide supply is less likely to become a limiting factor when carbonates are abundant.

NITROGEN. Nitrogen is essential to the synthesis of protoplasm, so that the amount of nitrogen available limits the amount of plant matter synthesized. Nitrogen also forms an important link in the chlorophyll molecule. Consequently nitrogen deficiency may result not only in a limitation of phytoplankton numbers, but also in a limited chlorophyll production and a yellowing of the cells (Chu, 1943; Rodhe, 1948). Einsele (1941) points out that the ratio of nitrogen to dry matter found in phytoplankton is about the same, whether the plankton comes from nitrate-rich or nitrate-poor media. Phytoplankters do not appear, therefore, to store nitrogen, but take up only sufficient for their immediate metabolic requirements.

Nitrate and ammonium compounds are generally considered to be available for plant metabolism (Rodhe, 1948). There is some evidence that some algae grow most efficiently when the nitrogen source is a nitrate, while others grow best with an ammonium source (Welch, 1952). Chu (1943) found that both ammonium and nitrate salts could be used directly by several species of diatoms and green algae which he cultured, but that the requirement might differ according to the source.

Table IV lists some of the limits of the "optimum ranges" of nitrogen concentrations found by Chu (1943). By "optimum range" Chu meant the range of nutrient concentrations between a lower limit, below which growth was negligible, and an upper limit, at which growth was maximal. The lower limit can be equated roughly to the lower lethal limit (i.e., to the starvation level). The upper limit is, however, of doubtful significance. As Chu did not raise the levels of other factors when he reached this upper limit of nitrogen concentration, there is no assurance that the levelling off in growth rate at this limit was really due to the nitrogen being at supra-optimal concentrations. His graphs suggest rather that this levelling off was due to some factor other than the nitrogen supply becoming limiting. A more exact interpretation of Chu's data seems to be that, under the conditions of his experiments, his lower limit indicates the minimal

nitrogen requirement of each species of alga, and that the nitrogen supply was a limiting factor, under the conditions of his experiments, from this lower limit up to at least the upper limit of his "optimum range".

TABLE IV. "Optimum ranges" of nitrogen concentration, as parts per million of N. Data taken from Chu (1943).

	Lower Limit	Upper Limit
Ammonium salt as N-source		
<i>Fragilaria crotonensis</i>	0.3	13.0
<i>Nitzschia palea</i>	1.3	6.5
<i>Pediastrum boryanum</i>	1.3	13.1
<i>Staurastrum paradoxum</i>	2.1	6.9
Nitrate salt as N-source and		
<i>Pediastrum boryanum</i>	0.14-0.69	13.9
<i>Staurastrum paradoxum</i>	0.85	more than 17.1
<i>Botryococcus Braunii</i>	0.35	6.9
<i>Asterionella gracillima</i>	0.51	17.0

At concentrations from about 25 ppm. upwards, Chu found that an increase in nitrogen concentration was accompanied by increased inhibition of phytoplankton growth. Since the original inoculum in media with more than 25 ppm. of nitrogen continued to live and grow, apparently without evidence of protoplasmic disorganisation, the inhibition here cannot be equated to a lethal effect. This inhibitory effect appears rather to be that shown by a controlling factor at supra-optimal concentrations. Thus a dual role is implied for the nitrogen supply, namely that of a controlling factor and a limiting factor. The explanation may be that, while the supply of nitrogen limits the rate of protein metabolism, the supply of nitrate or ammonium ions controls the rate of uptake of ions.

Although Chu's work does not indicate the actual upper limits of nitrogen tolerance for his phytoplankton, it does suggest that perhaps such an upper limit has little significance under natural conditions. A table in which he lists the concentrations of nutrient elements found in natural waters by numerous investigators indicates that these nitrogen concentrations range from a trace to 11.4 ppm., most of the values being less than 1 ppm. (Chu, 1942). On the other hand, his phytoplankton survived and grew in nitrogen concentrations up to at least 100 ppm. Chu (1943) suggests that, since the nitrogen concentration in ordinary waters is usually below 5 ppm. and seldom exceeds 13 ppm., it is improbable that the nitrogen concentration will have even an inhibitory effect on algal growth in these waters.

PHOSPHORUS. Pfeffer (1899) pointed out that "Phosphorus is essential [i.e., to plants], for it forms a constituent of many proteids, and nuclein contains as much as 6%". Since Pfeffer's time, phosphorus has been shown to play an important part in carbohydrate metabolism as well. The phosphorus supply may therefore affect both the building and the energy metabolism of plants (e.g., phytoplankton). In nature, plants obtain their phosphorus from phosphate salts.

Table V shows the limits of the "optimum range" of phosphorus concentration found by Chu (1943) for certain phytoplankton. As in the case of Chu's

TABLE V. "Optimum ranges" of phosphate-phosphorus concentration, as parts per million of P. Data taken from Chu (1943).

Nitrate salt as N-source and Phosphate salt as P-source	Lower Limit	Upper Limit
<i>Tabellaria flocculosa</i>	0.018	8.9
<i>Nitzschia palea</i>	0.018	8.9
<i>Pediastrum boryanum</i>	0.09	17.8
<i>Staurastrum paradoxum</i>	0.09	17.8
<i>Botryococcus Braunii</i>	0.09	17.8
<i>Asterionella gracillima</i>		17.8

nitrogen data, the lower limit here indicates approximately the minimum requirement for each species. A comparison of Tables IV and V shows that the minimal requirement of phosphorus is considerably less than that of nitrogen, under the same culture conditions. The minimal requirement for *Staurastrum paradoxum* and *Pediastrum boryanum*, for example, is about one-tenth the minimal nitrogen requirement (when the N-source is a nitrate). A lower phosphorus requirement (relative to nitrogen) was also found for certain blue-green algae by Gerloff *et al.* (1950b, 1952). Gerloff obtained good growth in cultures of *Microcystis aeruginosa* and of *Coccochloris peniocyctis* when the phosphorus concentrations were respectively one-seventy-fifth (i.e., 0.045 ppm. P : 13.6 ppm. N) and one-thirtieth of the nitrogen concentration.

Here again, the upper limit of the "optimum range" is of doubtful significance. Moreover, Chu gives no indication of the upper limits of tolerance, and his data suggest that the phosphorus content of natural waters is not likely to be large enough to have lethal or inhibiting effects on the phytoplankton. He shows, for example, that although the phosphorus content of natural waters ranges from a trace to 0.89 ppm., there was no inhibition of growth in his cultures at phosphorus contents of up to 8.9 ppm.

At concentrations of 45 ppm. upwards, the phosphorus supply apparently had an inhibiting effect on the growth of phytoplankton in Chu's cultures. Like the nitrogen supply, therefore, the phosphorus supply apparently has a dual role (i.e., limiting and controlling). This could be the case if the phosphorus supply limits the rates of protein or carbohydrate synthesis, while the concentration of phosphate ion affects the salt uptake.

Rodhe (1948) observed that the content of assimilable phosphorus in lakes "varies within an interval below 0.06 ppm.", and on the basis of his field and laboratory studies he classified phytoplankton according to their phosphorus requirements thus:

	Optimum	
	Lower limit	Upper limit
Class I. Low P requirement	less than 0.06 ppm.	less than 0.06
Class II. Medium P requirement	less than 0.06	more than 0.06
Class III. High P requirement	more than 0.06	more than 0.06

He suggested that the Chrysophyceae belong to Class I, the diatoms to Class II, and the green algae to Class III. This indication that the phosphorus requirement of green algae is higher than that of the diatoms agrees with Chu's data (cf. Table V). This high phosphorus requirement may perhaps account for the fact that, although green algae always appeared in the Algonquin Park lakes during the summer, they were abundant only when the nutrient supply (including phosphorus) was increased by fertilization (McCombie, 1950).

Since phosphorus can influence carbohydrate metabolism as well as protein metabolism, there is a possibility that the correlation between the phosphorus content of an environment and the production of plant protoplasm may in some cases be obscured. Einsele found, for example, that the phytoplankton in the Schleinsee and in cultures took up quantities of phosphorus far in excess of the amount reflected in population increases. He suggested that the algae incorporated large amounts of phosphorus into a mechanically transportable compound. Since the rate of phosphorus uptake depended upon the temperature and oxygen content of the water, the uptake must have been an "active physiological" process. Certain compounds which are involved in the energy metabolism (e.g., the phosphorylated sugars) might be just such compounds formed by "active physiological" processes.

The supply of available nitrogen and phosphorus in waters may affect phytoplankton production by (1) limiting the amount of plant matter that can be produced, and possibly also (2) restricting the floral composition to forms which can survive at N and P concentrations normally found in those waters. The eutrophication of lakes is closely associated with changes in the phosphorus and nitrogen supplies. The changes in phytoplankton populations which have accompanied the artificial eutrophication of lakes by domestic drainage have been discussed by Hasler (1947). Artificial eutrophication of lakes as a result of fertilizing has been reported by numerous investigators, and in the case of the Algonquin Park lakes, fertilization produced marked increases in abundance of chrysomonads, diatoms, green algae and blue-green algae.

POTASSIUM. According to Pfeffer (1899), "Potassium is presumably an integral constituent to protoplasm, and like nitrogen, phosphorus and also magnesium, is relatively abundant in embryonic tissues". Potassium is also abundant in growing (i.e., meristematic) tissues. Consequently this element is likely to be an important requirement of actively multiplying the phytoplankton. Scott (1943) points out that a deficiency of potassium ion in the medium leads to a cessation of cell division in cultures of *Chlorella pyrenoidosa*. He found that the cells tend to accumulate considerable amounts of potassium when it is plentiful, and to take up a chemically equivalent amount of sodium when potassium is scarce. Although this sodium apparently enables the cells to survive, it does not allow them to reproduce. Of the cations they studied (Mg, Ca, Fe and K) Gerloff *et al.* (1950b) found that potassium ion was required in greatest amounts for satisfactory growth of *Coccolithus Penicystis*.

Chu (1942) found that the potassium requirements of the phytoplankton he studied, like the nitrogen and phosphorus requirements, could differ markedly

for different species. He reported that the green alga *Pediastrum boryanum* produced heaviest crops when cultured at comparatively high concentrations of potassium ion. His diatoms on the other hand became most abundant in cultures having lower concentrations of potassium.

Although potassium is needed in culture media, and in fertilizers where the nutrient supply is being augmented, it may not in itself be a limiting factor for phytoplankton growth in natural waters. Potassium salts, when used alone, are not generally found to be efficient fertilizers (Neess, 1946). Rodhe (1948) states that "Concerning *Scenedesmus quadricauda*, I can confirm the common conception that magnesium and potassium should not be counted with the factors which limit algal development in lakes". This common conception has, however, been challenged by Vollenweider (1950), who feels that the importance of magnesium and potassium in phytoplankton production is too often underestimated.

Like the nitrogen and phosphorus supplies, the potassium supply probably plays the dual role of a limiting and a controlling factor. Indeed the ions of potassium, calcium and magnesium are known to control each other's actions in plant metabolism and growth (Vollenweider, 1950; and others). As this controlling effect seems frequently to be a modifying (or counteracting) one, potassium, calcium and magnesium are termed the "antagonistic elements". From his culture experiments Chu (1942) concluded that "The presence of excessive K renders the organisms [i.e., phytoplankton] tolerant to higher concentrations of Ca and Mg". It might be, however, that the presence of plentiful K ion actually enables the plants to utilize more Mg. Since Mg is a part of the chlorophyll molecule, such a dependence of Mg utilization on the K supply would help to account for pigment abnormalities attendant on K deficiency. Scott (1943) observed such pigment abnormalities in K-deficient cultures of *Chlorella pyrenoidosa*, and yellowing is a common symptom of K deficiency in higher plants. The potassium supply might therefore act as a controlling factor in chlorophyll synthesis, in that the potassium concentration would govern the rate at which magnesium is incorporated into the chlorophyll molecule, while the potassium itself would not become part of the molecule.

CALCIUM AND MAGNESIUM. The relation between the carbonate and bicarbonate salts of calcium and magnesium and the supply of carbon to phytoplankton has already been mentioned. Besides affecting the carbon supply, the presence of calcium and magnesium salts may also affect the pH of the environment. For example, these salts may prevent the pH from falling during times of vigorous organic decomposition by taking up the resulting excess of carbon dioxide. Hence the supply of calcium and magnesium carbonates and bicarbonates may be a *masking factor*, making it difficult to distinguish the effects of changes in carbon supply from those due to pH changes.

The effects listed in the preceding paragraphs are primarily effects of the carbonate and bicarbonates salts of calcium and magnesium, but there are other effects which are specifically due to the calcium and magnesium concentrations. Among these effects is the limitation of chlorophyll production by magnesium

scarcity. Rodhe (1948) found that cultures of *Scenedesmus quadricauda* in magnesium-free media turned yellow and eventually colourless within two weeks of inoculation. In contrast, cultures supplied with 1 ppm. of magnesium remained green much longer.

In addition to limiting chlorophyll production, calcium and magnesium may exert a controlling effect on certain functions of plant cells (e.g., by altering the permeability of the cells). Water retention is a function which is affected in this way. By affecting the permeability, the calcium and magnesium ions may affect the composition of the internal medium, and in turn control the rates of metabolic processes which depend on this internal medium for a supply of substrates.

The effects of calcium and magnesium ions on cell permeability are apparently opposed, so that calcium tends to decrease permeability and magnesium to increase it (Vollenweider, 1950; and others). If this difference in permeability is due to a competition between the ions for positions on the cell membrane (or on cell constituents), then one might expect the ratios of concentrations within (or on) the cell to be some function of the ratio in the environment. Scott (1943) found the ratio of calcium to magnesium in cells of *Chlorella pyrenoidosa* to be a linear function of their ratio in the culture medium. The uptake and utilization of calcium and magnesium by phytoplankton appear, therefore, to depend not only upon their absolute concentration in the environment, but also on the relative concentrations.

TRACE ELEMENTS. Certain other elements appear to be necessary for the healthy development of phytoplankton, but are required only in minute amounts. Some of these elements listed in the literature are: Li, Cu, Zn, B, Al, Ti, Sn, V, Cr, No, W, Mn, Br, Fe, Co, and Ni. The minute amounts of these elements which are sufficient to give a pronounced acceleration to plant metabolism suggests that in many cases they have a catalytic function (perhaps as units in enzyme structures). In this way the concentration of a trace element may be a *controlling factor* governing the rate at which the phytoplankton can utilize other elements.

Pringsheim (1946) and other pioneer culturists reported that many algae could be cultured only in media containing soil decoctions. While they were uncertain as to the function of such decoctions, they thought it likely that it concerned the supply of iron and possibly of other minor elements. The fact that Chu, Rodhe, Gerloff and others have since succeeded in culturing some of these algae in purely synthetic media, containing a suitable supply of iron and other trace elements, supports this assumption about the role of soil decoctions.

The importance of iron was further demonstrated by Rodhe (1948) when he improved Chu's No. 10 media by substituting ferric citrate and citric acid for the inorganic iron salt used by Chu. With Rodhe's modification the iron remains in solution longer. A deficiency of iron results in poor growth and chlorophyll production in phytoplankton cultures (Rodhe, 1948). Rodhe found, however, that the addition of as little as 0.0015 gm. of iron per litre of medium to iron-starved cultures of *Scenedesmus* had an immediate beneficial effect on the growth and chlorophyll formation. The exact biochemical role of iron in chlorophyll production is unknown. If, as some investigators suggest, an iron porphorin is a

precursor of chlorophyll, then an iron deficiency may limit chlorophyll synthesis by limiting the production of this intermediary. On the other hand one might speculate that the effect of an iron deficiency is manifest in the energy metabolism through the cytochrome system. Whatever the biochemical role of iron may be, there is little doubt as to its importance to phytoplankton.

Unfortunately, little appears in the literature about the function of other trace elements in the metabolism of plankton algae, apart from the statement that lithium, copper and zinc are apparently necessary. Since different investigators have used several combinations of these minor elements satisfactorily, it seems that different species have different requirements and that these elements may substitute for each other to some extent. Ström (1933) reported that manganese could be substituted for iron in cultures of *Chlamydomonas ventralis* and possibly also of *Coccomyxa simplex*.

It is generally agreed that concentrations of the trace elements in excess of the minute amounts required by the phytoplankton may be toxic. Unfortunately there is little if any precise data on the tolerances of plankton algae to these elements, but the use of copper sulphate as an algicide depends upon the toxicity of this element at relatively low concentrations.

SPECIAL NUTRIENT REQUIREMENTS. Besides the general nutrient requirements of phytoplankton discussed above, there may be some elements which are of special importance to particular forms of algae. Silicon appears to be such a special requirement. The supply of this element is of special importance to diatoms, since these organisms incorporate large amounts of silicon in their skeletal structure. Pearsall (1923) was among the first to suggest that seasonal changes in the silicon content of waters, which are associated with changes in turbidity (i.e., of silt content), may be correlated with diatom production. According to Chandler (1942) diatoms compose a greater percentage of the total standing crop of phytoplankton in western Lake Erie when the turbidity exceeds 25 ppm. than when it is less than 20 ppm. He found that the turbidity was correlated partly with the disturbance of bottom sediments by greater wind stirring during the spring and autumn. However, ions other than silica may also be made available at the same time. Spencer (1950) thought that silicon might become a limiting factor to diatoms in the Quabbin Reservoir, since the content of soluble silicates decreased as diatom pulses developed but not concurrently with the development of pulses of other phytoplankton.

HYDROGEN ION CONCENTRATION

The hydrogen-ion concentration in the aquatic environment may act on phytoplankton as a *controlling factor* or as a *lethal factor*, according to its level. One might speculate that the pH acts as a controlling factor by governing the rate of some enzyme activity within the algal cell. The lethal effect will be manifest when the pH reaches a value outside the pH-tolerance limits for the alga. Some algae appear to be adapted to alkaline conditions, while other species flourish under acid conditions.

Unfortunately the role of pH in the metabolism of phytoplankton has been subject to few detailed laboratory investigations. Gerloff *et al.* (1950b) demonstrated that the blue-green alga *Coccochloris Peniocystris* requires a high pH for maximal growth. There was no growth in cultures kept at pH 5.0 and 6.0, and only slight growth at pH 7.0 and 8.0. The growth was considerably greater at pH 9.0, was maximal at 10.0 and declined again at 11.0. The pH-tolerance range of this alga is therefore towards the basic end of the pH scale, and optimal growth occurs in quite alkaline conditions. Gerloff *et al.* have also cultured several other species of blue-green algae successfully at relatively high pH's, but their studies on these species were less detailed.

Many field investigations indicate that the range of pH characteristic of a lake may determine which particular species of algae will be present in it. Thus, Prescott (1951) points out that a luxuriant desmid flora is typical for soft-water lakes in Michigan and Wisconsin, whereas hard-water lakes in those states are characterized by a cyanophycean-diatom flora. These floral types correspond respectively to the Caledonian and Baltic floras described earlier by Smith (1938) and others. In the case of some algae, the pH tolerance is so narrow that their presence in a body of water can be used as an indication of its pH. For example, Prescott (1951) says of *Anabaena flos-aquae*: "The occurrence of this species is so consistently related to hard water lakes that it may be used as an indicator organism for high pH . . ." In contrast, *Nitella* is a genus almost always confined to soft waters which are rich in humic acids.

The influence of pH on the growth and survival of plankton algae is particularly complicated, owing to the fact that the pH of the environment depends upon the levels of other factors. Consequently, the correlation reported between high phytoplankton production and hardness of waters may in some cases be a correlation of production with high calcium and magnesium supply, rather than with high pH. Moreover, it is well known that a heavy growth of phytoplankton can raise the pH of the environment when the increased photosynthetic demand reduces the carbon dioxide content of the water. However, this correlation between the numbers of algae and the pH should be distinguished from that between the kind of algae and the pH made in the paragraph immediately preceding. The quantity of phytoplankton may be determined chiefly by the controlling effect of pH, whereas the quality probably depends more upon the lethal effect. That is, the numbers will depend on how near the pH is to the optimum of the algae present, while the species present will be forms which can survive at the existing pH.

ACCLIMATION

The extent to which phytoplankton responds to a change in the level of an environmental factor may depend upon the level of that factor to which the algae have become acclimated. Up to the present the phenomenon of acclimation in phytoplankton has been demonstrated for temperature and for light intensity. Further investigations may reveal that they can become acclimated to other factors (e.g., hydrogen-ion concentration) as well.

Harvey (1939) showed that the optimum light conditions for growth of the diatom *Biddulphia mobiliensis* depended to a marked degree upon the conditions under which it had lived prior to his experiments. He divided a culture of this diatom into two parts. One part was exposed to dim December daylight. The other part was continuously illuminated at 18,000 lux (1,680 f.c.) by a light bulb. At the end of a week subcultures were made and kept at different temperatures and light intensities. After 72 hours the increase in the number of diatoms in each vessel was determined (Table VII).

TABLE VII. The effect of acclimation on the response of *Biddulphia mobiliensis* to different conditions of light intensity and of temperature. After Harvey (1939).

	Temp. °C.	Percentage increase in 72 hrs. at				
		28000	18000	8000	4100	1400 lux
Cells grown previously	18	7	68	66	106	87
in dim daylight	13	24	16	14	8	2
Cells grown previously in continuous	18	171	236	190	123	98
light, at 18,000 lux	13	31	70	105	67	36

The data show that the response to changes in both light intensity and temperature differed with the conditions under which the parent stocks were kept.

Myers (1946) demonstrated that the extent to which the photosynthetic rate of *Chlorella* increases with increasing light intensity depends upon the light intensity at which the cells are originally cultured. (See also under heading of the intensity of light.) Myers's graphs show that both the rate at which the photosynthetic activity increases with increasing light intensity and the level at which light intensity ceases to be limiting, vary with the acclimation levels of light intensity.

The phenomenon of acclimation has been too often overlooked. In future more care should be taken to describe the conditions under which the phytoplankton is kept prior to experiments. This is particularly desirable if the resultant data are to have absolute values, and if the data of different investigators are to be pooled.

BIOCHEMICAL FACTORS

In addition to the physical and chemical factors influencing the growth of phytoplankton, there are at times biochemical factors which arise from the metabolism of these organisms. Such factors are the concentrations of autotoxins and antibiotics. The chief effect of these factors is apparently to inhibit the growth of organisms. In this role, the concentrations of autotoxins and antibiotics are probably *controlling factors*. This control might conceivably be the result of these substances altering the pH of the environment, or competing with a normal substrate in some metabolic process.

AUTOTOXINS

It has frequently been suggested, and in a few cases demonstrated, that organisms in culture synthesize metabolic products which eventually prevent

further growth of the organisms themselves (Robertson, 1923; and others). Such substances are the autotoxins. Pratt (1940) discovered that, during early growth stages of *Chlorella vulgaris*, cultures with the least dense population had the highest growth rate. The rate of reproduction per cell steadily decreased as the population increased. Furthermore, in a series of cultures inoculated with different numbers of cells, the rate of multiplication throughout the growth period varied inversely with the initial population density. Pratt interpreted this to mean that the cells released a growth-inhibiting substance into the medium. It seems unlikely that the decreased growth in the denser cultures was due to exclusion of light, because the differences in growth were noted even at early stages before the concentration of cells could appreciably affect the light penetration. Pratt proposed the name *chlorellin* tentatively for the inhibitor secreted by *Chlorella*, and in further investigations (Pratt, 1942, 1943) he determined some of its physical and chemical properties. By culturing *Chlorella* in collodion sacks immersed in a nutrient medium, Pratt (1944) was able to show that, when the medium was renewed or was filtered through carbon, the final population density obtained was about three times that when the medium was not renewed or filtered. The fact that the treatment with carbon had an effect similar to renewing the medium makes it unlikely that the slowing up of growth resulted from a reduction of nutrients. That is, the carbon apparently removed something harmful but did not alter the nutritive value of the medium.

ANTIBIOTICS

In some cases phytoplankton may synthesize antibiotics which inhibit the growth of other organisms. Lefèvre *et al.* (1948) filtered cultures of algae and then inoculated media containing these filtrates with other species. There was a complete lack of growth in some cases, or an inhibition in others. They argued that this inhibition was not due to exhaustion of nutrients, since satisfactory growth occurred in a medium which was eight times poorer in mineral content. Appreciable growth took place if the filtrate was diluted with distilled water, or if it was boiled. Lefèvre found that the filtrate from cultures of *Scenedesmus quadricauda* appeared to have only an inhibitory effect on *Pediastrum Boryanum*, and on several other green algae. Filtrate from cultures of *Pandorina morum* seemed to have a dual effect on the same species, namely it caused a marked increase in reserve materials and it inhibited cell division.

Upon further investigation, Lefèvre *et al.* (1950) suggested that the secretion of antibiotics by dominant algal species might be an important factor in bringing about the almost unispecific composition frequently reported for water blooms. They found that filtrates of waters from canals in which there were blooms of *Aphanizomenon gracile* or *Oscillatoria planctonica* inhibited growth in cultures of *Cosmarium Lundelli* and other plankton algae. However, the antibiotic property diminished quickly with age, so that the antibiotic probably deteriorates rapidly after blooms decline.

The production of autotoxins and antibiotics must therefore be considered in both field and laboratory studies. The danger that the action of these substances

may obscure the effects of other environmental factors is, of course, greater in cultures where the volume of the environment is relatively small.

CONCLUSIONS

In lakes in many parts of North America there is a cyclic succession of pulses of different algal species throughout the year. This phenomenon is apparently due largely to the cyclic change in water temperature which depends ultimately upon the seasonal change in solar radiation. In many cases, however, this cyclic succession of pulses may be obscured to some extent by irregular changes in phytoplankton numbers resulting from local weather or nutrient conditions. Weather conditions may affect both the degree and time of development of an algal pulse. Changes in nutrient conditions, on the other hand, seem to affect primarily the degree of development. Thus a period of exceptionally warm weather may cause the spring diatom pulse to develop earlier in a particular case, but an increase in the nutrient supply (e.g., by fertilization) will not cause the pulse to develop before the water temperature is suitable. The increase in nutrient might nevertheless cause the pulse to be heavier than usual ultimately.

The conditions of climate, weather and nutrient supply can be resolved into the component factors of light intensity, duration of illumination, water temperature, concentrations of nutrient elements, ionic balance, pH, etc. These factors can in turn be classified according to specific effects on the phytoplankton as limiting, controlling and lethal factors. Limiting factors are those which affect the metabolic rate through the supply of energy or materials. The light intensity, duration of illumination and concentration of nutrient elements are limiting factors. Controlling factors are those which govern the rate of metabolism and activity but are not concerned with the supply of energy or materials. The temperature, ionic composition and pH are controlling factors. Growth-inhibiting substances which affect the rates of metabolism and growth are perhaps controlling factors also. At certain concentrations many of these factors may become lethal factors affecting the survival of the phytoplankton. The abundance of a particular species of alga at a given time probably depends largely on the extent to which the limiting and controlling factors favour its growth. What species will be present at that time probably depends more on the lethal factors. That is, species for which the concentration of any factor is toxic will be excluded. The terms limiting, controlling, inhibiting, lethal have sometimes been used rather loosely in the literature on phytoplankton, but it should be realized that these terms can and should have a precise meaning (Blackman, 1905; Fry, 1947).

The degree to which the phytoplankton responds to changes in the level of any environmental factor may depend upon the level of this factor to which the phytoplankton is acclimated. The importance of acclimation is too often overlooked. It is possible that in many cases the degree of acclimation determines whether a change in the level of a factor is too great or too rapid for the metabolism of the phytoplankton to become adapted. Consequently the growth and survival of the algae in a changing environment, such as a lake, may depend not only upon the degree of change but also on the rate. It is particularly important

that the previous history of plankton stocks used in laboratory experiments be given. Otherwise, data on optimum, limiting and lethal levels of environmental factors can have only a limited significance.

There is need for more caution in interpreting data from culture experiments. Many investigators give too little consideration to the possibility that their results may have been affected by exhaustion of nutrients or accumulation of autotoxins. Some investigators, however, attempt to avoid the effect of changes in the medium by taking the growth rate at the logarithmic phase as an index of the limiting effect of the factor under test. The best culture method would, of course, be one where the medium was frequently or continuously changed.

Finally, although the growth of the phytoplankton depends upon the physical and chemical conditions in natural waters, the actual numbers of algae found there can be markedly influenced by cropping of zooplankton (Fleming, 1939; Pennington, 1941; Riley *et al.*, 1946a and b, 1949) or by fungal parasitism (Canter and Lund, 1948). These factors have, however, been omitted from the present discussion because the conclusions drawn here from field observations are supported in general by studies of algae in cultures free from predators or parasites.

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The Mineralization of Plankton

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ABSTRACT

The rate and the thoroughness of the decomposition of plankton remains are important factors in the cycles of some nutrients in lakes. Experimental data on the mineralization of plankton *in vitro* are available, especially of marine organisms, but quantitative observations on this process in nature are not mentioned in the literature. This paper presents the results of such observations, made in Lake Lauzon, Montcalm County, P.Q., by means of a sediment collector described in an earlier paper. The chemical composition of plankton, of sediment collected at the 11-meter level, and of surface bottom sediments are studied. Most of the decomposition of the sinking detritus takes place in the epilimnion of the lake. Sediment collected at the 11-meter level, in weekly intervals, shows a remarkable resemblance in composition with the surface bottom sediments. Most of the nitrogen is liberated in the upper 11 meters of water. The liberation of phosphorus seems to be slower and the surface bottom sediments are actually enriched with this element. Silica is enriched at both the 11-meter level and the bottom of the lake, mainly through the settling of diatom frustules. Hydrolysis of organic matter in the upper 11-meter column of the lake decreases in the course of the summer. Mineralization seems to be practically halted in the oxygen-poor hypolimnion.

INTRODUCTION

FOR MANY YEARS limnologists have tried to arrive at a better understanding of the mechanisms of environmental factors which determine biological productivity in the lakes. Biological productivity, when referred to in this paper, will mean the amount of organic matter which the lake is able to produce in a certain unit of time.

It was early recognized, of course, that the basic source of production of organic matter is the photosynthetic process carried out by the green plants in the lake. Therefore, optimum productivity requires optimum conditions for photosynthesis: light, temperature and nutrients. Both theoretical limnologists and those primarily interested in applied limnology, such as fisheries biologists, became interested in the determination of the absolute productivity of lakes as well as in the environmental factors which, alone or in combination with others, establish the level of productivity. Quantitative plankton studies, chemical analyses of the water and of the plankton, qualitative and quantitative bottom fauna studies, observations on the transmission of solar energy and of thermal conditions have all contributed to a better understanding of this very complex problem. Thiennemann's classifications of lakes with respect to their productivity were particularly stimulating and were based mainly on concentrations of dissolved oxygen and on the composition of bottom fauna populations. Naumann's studies on lake bottom sediments emphasize especially the relationships between productivity and lake pedology.

In the present paper emphasis will be laid on certain aspects of the cycles of nutrients in so far as these are affected, according to our present knowledge, by the processes of mineralization of plankton. These aspects have not received a great deal of attention on the part of limnologists, perhaps because of the technical difficulties involved in their study. It is obvious, however, that the decomposition or "mineralization" of plankton is of fundamental importance in the whole concept of biological productivity. Perhaps this can best be illustrated by taking, for example, a hypothetical lake, supplied with dissolved inorganic nutrients through ground water, neither enriched by allochthonous materials nor impoverished by losses through outlets, fishing or other causes. In such a lake the nutrient elements required by the phytoplankton are present in the form of solutes, as well as in the bodies of producers, consumers, reducers and organic detritus in the sediments and bottom substrates. Among the producers, phytoplankton is the most important, whereas the consumers graze on the organic matter, alive or dead, synthesized by the producers. The reducers, mainly bacteria and protozoa, destroy dead organic matter directly or indirectly derived from producers. Especially, the work of the consumers and the reducers leads to the hydrolysis of the organic matter and the liberation of mineral nutrients, which may again be used by the producers.

If, in our hypothetical lake, these processes of synthesis and destruction were perfect ones, eventually all of the nutrients used by the producers in the formation of organic matter would again become available for new generations of producers. Therefore, our lake would have to develop into a eutrophic lake within a relatively short time, because the steady supply of dissolved nutrients through the ground water would enrich the environment, whereas no losses would occur. From this example it can be inferred that the critical phase in the eutrophization of the lake, in so far as the availability of nutrients is concerned, is to be sought in the rate and the degree of liberation of nutrients from the organic matter contained in the bodies of producers, consumers and reducers and their waste products. In a lake with a perfect and complete cycle, all nutrients or organic detritus would eventually be decomposed and transformed into mineral substances. In other words, no organic detritus would accumulate permanently in the lake-bottom sediments and no nutrients would be permanently removed from their cycles. Therefore, no minerals would be added to the lake-bottom materials.

There is no such lake in nature, although it is possible that some lakes approach our hypothetical type rather closely. Our example, nevertheless, serves the purpose of emphasizing the importance of mineralization or decomposition of organic matter, specifically that contained in plankton, in the economy of nutrients, and, therefore, in the biological productivity of the lake. Barring very exceptional geological conditions, lakes supplied by ground water will be able to produce a phytoplankton crop, however small, provided adequate light and temperature conditions prevail. As even this very small phytoplankton population would eventually lead to eutrophization, according to our previously mentioned example, it seems that much of the problem of eutrophization of lakes is hinged

on the intensity and thoroughness of the processes of mineralization or regeneration of decaying plankton. It is conceivable that a lake supplied with a small amount of mineral nutrients, but characterized by intensive mineralization of the dead organic matter could be more productive than another lake, well supplied with minerals but having a sluggish and very incomplete mineralization of its organic matter.

In nature, the decomposition of organic matter is rarely complete. Even on dry land, given proper conditions of aeration, humidity and chemical composition, organic matter produced by higher plants is rarely, if ever, decomposed completely into its original "building stones": minerals, carbon dioxide and water. For the establishment of terrestrial vegetation this is quite fortunate, because the remaining organic matter is the indispensable component of top soil, allowing for a development of a beneficial microbiotic population in the soil, and otherwise favouring conditions for the growth of terrestrial vegetation. The degree to which organic matter is mineralized in the terrestrial environment depends on the nature of the organic matter, climatic conditions (primarily temperature and humidity), and the availability of certain minerals like calcium. The prevailing redox potentials in the organic matter determine, to a great extent, the degree to which organic substances can be hydrolysed by bacterial enzymes or decomposed by abiotic chemical processes. As a rule, however, due to the availability of oxygen, decomposition of organic matter on dry land is rather complete and rapid.

Turning now to the decomposition of organic matter in lakes, two main differences, as compared with conditions found on land, are striking. These are (1) the chemical composition of the plankton, and (2) the reduced availability or even periodic or permanent absence of oxygen in the hypolimnion. With respect to the chemical composition, it may be stated that, with rare exceptions, organic matter derived from lake plankton has a considerably higher content in proteins and fats than that derived from terrestrial vegetation. This difference is clearly seen in the chemical composition of bottom sediments in eutrophic lakes in which a considerable part of the sediment is made up of partially decayed remains of plankton (Naumann's "sapropel"). Of greater importance in the processes of mineralization of plankton are the above-mentioned oxygen conditions which, at least in eutrophic lakes, temporarily or permanently make impossible the establishment of favourable redox potentials in the uppermost layer of the bottom sediments which, in such lakes, typically consist of plankton remains ("ävja" of the Swedish authors).

That mineralization of plankton is very incomplete in most lakes can be inferred from the chemical composition of the upper lake-bottom sediments which almost invariably, even in extremely oligotrophic lakes, contain considerable amounts of nutrient elements in the form of organic compounds resistant to decomposition under the prevailing environmental conditions. It has been supposed that these materials are mineralized in bottom sediments to a considerable degree and that dissolved nutrients, with or without intervention by bottom organisms (Alsterberg, 1924), become available in the trophogenic layer of the

lake on the occasions of total overturns. This supposition has not been borne out by chemical analyses of lake-bottom sediments.

Observations in lakes of the Precambrian of the Province of Quebec (Kleerekoper and Grenier, 1952) and of the Ordovician of Ontario (Kleerekoper and McGibbon, 1952) show a considerable accumulation of critical nutrients like nitrogen and phosphorus in these sediments. This is not surprising in view of laboratory experiments on the decomposition of plankton in aerobic and anaerobic conditions (Waksman *et al.*, 1933). Regeneration of nutrients is practically halted in anaerobic conditions which we know to prevail in the bottom sediments of all eutrophic and of many oligotrophic lakes investigated on this point. Even when the surface film is aerobic, settling organic detritus, especially during periods of high plankton production, will rapidly cover the surface of the sediment with a fresh layer of decaying substances ("ävla"), producing anaerobic conditions underneath. It seems, therefore, that a considerable part of the organic detritus incorporated in the bottom sediments of a lake is a net loss for the nutrient economy of the lake. Mineralization which does not take place in aerobic regions of the lake, especially in the trophogenic layer, will most likely not take place at all in the surface bottom sediments in anaerobic conditions. Our own observations, carried out with the collaboration of Mr. F. Grenier, chemist of the Biological Bureau of the Province of Quebec, seem to support this view (see below). To what extent Alsterberg's views are correct with respect to the role of bottom fauna in the regeneration of nutrients from organic matter will have to be further investigated through chemical analysis. It is likely that coprogenic materials are more readily decomposed than the original sediment ingested by the animals.

In spite of the importance of mineralization of plankton in lakes, no reports on observations *in situ* are to be found in the literature. There are, however, a number of reports on experiments dealing with the decomposition of plankton and the regeneration of nutrients *in vitro* and under laboratory conditions. The marine biologists especially have given much attention to these problems, and their findings are enlightening, although the artificial conditions of nutrition, temperature and surface interaction under which these experiments had to be carried out, show what could happen rather than what really occurs under conditions of the natural environment (Von Brand *et al.*, 1937; Waksman *et al.*, 1933; Cooper, 1933, 1935).

According to theory, proteins and other nitrogenous compounds in plankton bodies, when attacked by the enzymes of bacteria in the course of the processes of decay, should subsequently be broken down into albumose, peptones, amino acids, nitrogen-free acids, ammonia, carbon dioxide, water, and hydrogen sulfide. It is doubtful whether all of these intermediate "breakdown" products were ever found under experimental conditions. Brand, Rakestraw and Renn (1937), studying the regeneration of nitrogen in decaying plankton, were unable to detect intermediate compounds and concluded that NH_3 is produced directly from the decaying proteins. Cooper (1937), on the other hand, was able to show intermediates like amino acids, amines, purines and urea. It seems that the NH_3

produced is quickly oxidized by photochemical, chemical and bacterial oxidations. Other observations (Von Brand *et al.*, 1937) refer to the formation of NO_2 in decaying plankton, apparently in the absence of nitrifying bacteria. Waksman, Carey and Reuszer (1933) studied the rate of nitrogen liberation in decomposing plankton and observed that one-half of the total nitrogen was liberated in the form of NH_3 in the course of 19 days at a temperature of 16° to 20°C . Only one-fifth of the total carbon had been liberated as CO_2 at the end of that period.

According to Cooper's findings (1935), the regeneration of phosphorus from zooplankton occurs at a more rapid rate than that from phytoplankton. Diatoms are very resistant to bacterial decomposition, but will decay more readily after having been attacked by protozoa (Waksman, Stokes and Butler, 1937). Cooper found that all the phosphorus present in zooplankton had been liberated in the course of two weeks, whereas in phytoplankton the regeneration was still incomplete after five months. The same author also believes that the regeneration of phosphorus is more rapid than that of nitrogen. Redfield and co-workers (1937) made observations on the decomposition of plankton in the Gulf of Maine and concluded that regeneration of phosphorus takes place through the column of 240 meters of water. Later observations by the same author (1942) show that most decomposition in tropical seas occurs above the layer of minimum oxygen. Cooper (1935) made observations on the regeneration of phosphorus in diatoms in the dark. Only two-thirds of the organic phosphorus had been regenerated in the form of P_2O_5 after a period of three months. Both Cooper (*ibid.*) and Seiwel and Seiwel (1938) observed that the regeneration of phosphorus in decaying plankton was rapid at the start but slowed down considerably afterwards. Cooper noticed a second liberation following this lull. The above results of some of the observations published give interesting information on the processes of decomposition of plankton *in vitro*. Only Redfield's observations were made in the natural environment and deal with conditions found in the sea. Few or no data have become available on similar processes of plankton decay in fresh water.

EXPERIMENTAL

Because of the importance of mineralization of plankton in lakes as related to the problems of biological productivity, the author started a number of observations on the decay of plankton in Lake Lauzon, Montcalm County, Province of Quebec, in the summer of 1949. These observations were part of an extensive limnological study of that lake made under the author's direction. The investigations were carried out from the Biological Station of Mont Tremblant and under the auspices of the Biological Bureau of the Province of Quebec.

There is little doubt that the decay of plankton remains in lakes is brought about mainly by the action of bacterial enzymes. Its quantitative and qualitative aspects must, therefore, be closely related to the quantitative and qualitative aspects of the bacterial flora which are themselves dependent, to a great extent, upon the nature of the organic matter available for their nutrition. It is obvious that these intricate relationships and other dynamic equilibria cannot be reproduced *in vitro*. Only observations carried out in the lake itself can lead to informa-

tion as to what actually happens in the natural environment. In order to make quantitative and qualitative observations on the decay of descending plankton organisms in the lake, a special collecting apparatus was constructed as described elsewhere (Kleerekoper, 1952). By means of this apparatus, descending detritus can be collected quantitatively in the water on a 1-sq. meter tray at any desired depth in the lake.

On July 2, 1950, the apparatus was suspended from a buoy in the deepest part of Lake Lauzon (about 27 m.) at a depth of 11 meters, just below the thermocline. It was closed and brought to the surface for the collection of the sediment on August 7, 1950. During the above period of 35 days, .2154 grams of dry material were collected on the 1-sq. meter surface of the apparatus. The average standing crop of plankton in the 11-meter column above the apparatus during the summer period was 6.49 grams. The standing crops was determined as follows: One thousand to 1,500 liters of water were pumped, at one-meter intervals, from the zero level to the base of the eleven-meter column and filtered through a plankton net of No. 20 bolting silk. The dry weight of the material collected was multiplied by 5.5 in order to obtain the values for total centrifuge plankton. The factor 5.5 was obtained by comparison of values for centrifuge plankton and net plankton, simultaneously, on a number of occasions at the same levels. On September 25, 1950, the sediment collector was again lowered to the 11-meter level, where it remained throughout the following winter and early spring. It was brought to the surface again on July 6, 1951, when the sediment collected was removed for chemical analysis. In Table I, the average chemical composition of the standing plankton crop and the chemical composition of the sediment collected at the 11-meter level are shown. For comparison, the average chemical composition of a large number of surface bottom sediments (Kleerekoper and Grenier, 1952) is also entered in the table.

In the summer of 1951 the sediment collector was again suspended at the 11-meter level. The sediment collected on the apparatus was recovered at weekly intervals between July 11 and August 15. Average standing crops of plankton in

TABLE I. The average chemical composition of plankton, detritus settled at the 11-meter level, and surface bottom sediments, in Lake Lauzon, Montcalm County, P.Q.

Item	Plankton average 1951	Sediment at 11-meter level		Surface bottom deposit
		July 2 to Aug. 7, 1950	Sept. 25, 1950 to July 6, 1951	
Loss on ignition	% 94.3	% 52.6	% 59.7	% 57.8
lignin	25.6	20.3	22.5	26.3
N	7.37	1.98	2.68	1.24
P	0.187	0.133	0.181	0.22
SiO ₂	1.17	36.4	28.0	32.7
Ca	0.26	0.52	0.48	0.56
Mg	0.26	0.24	0.11	0.36
Fe	0.36	2.16	..	1.16

the 11-meter column were determined and chemically analysed, together with the sediment collected at the 11-meter level. The results of the observations are presented in Table II. In this table, the chemical composition of the sinking detritus is represented in weight percentages of the total amounts of the various chemical substances found in the standing plankton crop, and the data in the "plankton" column indicate the total weights of the various substances present in the 11-meter column of water above the sediment collector. In the "sediment" column the chemical composition of the sediment at the 11-meter level is entered as percentages of the amounts found in the plankton.

TABLE II. The chemical composition of the average total standing plankton above the sediment collector and of the sediment collected at the 11-meter level during five one-week periods in 1951, Lake Lauzon, Montcalm County, P.Q. (Plankton is total standing crop in 11-meter column above sediment collector. The chemical composition of the sediment in the collector is expressed as percentages of the amounts present in the total standing plankton crop in the 11-meter column.)

Item	July 11-18		July 18-25		July 25-Aug. 1		Aug. 1-8		Aug. 8-15	
	Plank- ton	Sedi- ment	Plank- ton	Sedi- ment	Plank- ton	Sedi- ment	Plank- ton	Sedi- ment	Plank- ton	Sedi- ment
Dry weight	g.	%	g.	%	g.	%	g.	%	g.	%
Loss on	7.077	33	6.777	48	7.210	47	6.688	66	6.385	69
ignition	7.238	16	6.270	21	6.633	25	6.160	39	5.780	39
N	.671	7	.577	11	.693	7	.555	15	.528	22
P	.050	0.7	.044	12	.060	9	.049	22	.044	12
SiO ₂	.132	762	.115	1148	.770	1642	.187	765	.121	1081
Ca	.055	30	.049	33	.044	25	.022	100	.011	150
Mg011	150	.016	100	.011	150

DISCUSSION

The data of Table I suggest that the sinking organic matter of the plankton was subjected to intensive oxidation processes, resulting in a degree of loss on ignition of from 94.3 to 52.6 per cent during the period July 2-August 7. As might be expected, this decomposition is less rapid during the fall and winter, as is shown by the higher loss on ignition during that period (59.7 per cent). In comparing the loss on ignition of the material collected at the 11-meter level with that of the average surface bottom deposits, the similarity of the figures is striking (52.6-59.7-57.8 per cent). This means that the bulk of the decomposition of the sinking detritus takes place in the epilimnion of the lake. This point of view is further supported by the other data on the chemical composition of the three types of material presented in this table and by the data of Table II. It is recalled that Redfield (1942) showed, for the tropical area of the Atlantic, that most decomposition takes place above the layer of minimum oxygen. For Lake Lauzon, oxygen depletion and accumulation of H₂S in the hypolimnion are characteristic.

The difference in total nitrogen between the plankton sediment at the 11-meter level, and that of the lake bottom (7.37-1.98-1.24 per cent) would indicate that most of the nitrogen present in the plankton was liberated in soluble form in

the 0- to 11-meter column. It is most likely that the nitrogen remaining at the 11-meter level and in the bottom deposits is in the form of proteins and complexes of humin acids, more highly resistant to decomposition.

The events relating to the decomposition of the phosphorous compounds do not become any clearer from the data presented in Table I. Apparently a certain amount of liberation of phosphorus does take place in the upper 11-meter column, especially during the summer period (plankton, 0.187 per cent; sediment at 11-meter level, 0.133 per cent). The higher percentage found in the bottom sediments (.22 per cent) is probably due to ferric phosphate precipitation, but further investigation will be required to settle this point.¹ The large amount of silica oxide at the 11-meter level and in the bottom deposits is striking. Diatom frustules were abundant in the material collected at the 11-meter level, as well as in the average lake bottom sediments (see Kleerekoper and Grenier, 1952). The presence of these frustules explains, in part, the high silicate content of these materials. Birge and Juday (1922) reported 30.78 per cent silicate oxide in the diatoms of Lake Mendota. The high resistance to decomposition of diatom frustules, as compared with the other substances in the sinking detritus, further explains the high silicate oxide content at the 11-meter level and in the lake-bottom sediment. It is important to note that sand grains were not observed in the microscopic preparations of the material collected by means of the sediment collector. In this connection, it may be recalled here that Lake Lauzon is completely surrounded, down to the water's edge, by dense forest.

The higher amount of calcium at the 11-meter level, as compared with that of the plankton, is due mainly to the higher loss on ignition of the latter. There seems to be, however, a slight enrichment of calcium in the bottom deposits.

It seems evident from the data in Table II that the rate of decomposition of the descending detritus decreased during the course of the summer. Whereas in the week of July 11-18 the loss on ignition of the 11-meter sediment was only 16 per cent of that of the plankton, this figure had increased to 39 per cent in the week of August 8-15. This decrease in rate of decomposition is further shown by the increasing amounts of nitrogen in the detritus at the 11-meter level (July 11-18, 7 per cent; August 8-15, 22 per cent).

It is difficult to venture an explanation for the decreasing rate of decomposition of the sinking plankton observed during the summer. The chemical composition of the plankton did not change noticeably, so that the nature of the decaying material does not seem to be a factor. Somehow, the bacterial activity responsible for the hydrolysis of the organic matter must have decreased in the course of the summer. Which of the environmental factors was responsible for this was not determined. It is of particular interest in this connection that Cooper, Murray and Kleerekoper (MS) were able to show by experiment that an increased availability of phosphorus considerably increased the bacterial activity in bottom sediments of Lake Lauzon. Experiments and observations are being continued to

¹Recent results of investigations by the author and his students on the organic components of lake bottom sediments in the Ordovician and Post-Ordovician of southern Ontario indicate that a considerable proportion of the phosphorus is in the form of water soluble protein.

obtain further information on this important aspect of mineralization of plankton in Lake Lauzon.

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